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GROWTH AND SWIMMING ENDURANCE OF JUVENILE CHANNEL CATFISH
IN HIGH TEMPERATURE ENVIRONMENTS

By

Michael Brigham Arnold

A Thesis
Submitted to the Faculty of
Mississippi State University
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for the Degree of Master of Science
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in the Department of Wildlife, Fisheries, and Aquaculture

Mississippi State, Mississippi

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GROWTH AND SWIMMING ENDURANCE OF JUVENILE CHANNEL CATFISH
IN HIGH TEMPERATURE ENVIRONMENTS

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Channel catfish (*Ictalurus punctatus*) are important to aquaculture and natural ecosystems, however little is known regarding effects of high summer temperatures, which are predicted to increase with climate change. Therefore, two studies were conducted to examine physiological effects of high temperatures on juvenile channel catfish. The first examined effects of three cycling thermal regimes (23-27°C, 27-31°C, and 31-35°C) characteristic of culture environments in Mississippi on growth, food consumption, feed conversion ratio, specific growth rate, and activity. The second study measured active and resting metabolic rates and swimming endurance at constant temperatures (27, 31, and 35°C). The best growth and feed conversion occurred at 27-31°C and activity was greatest at 31-35°C. Active metabolism and swimming endurance decreased at 35°C. These results indicate high summer temperatures reduce food consumption, increase activity, impair metabolism and endurance, and therefore present challenges to the culture and management of channel catfish.

DEDICATION

The completion of this thesis would not have been possible without the boundless love and support from my parents, John and Tracy. Furthermore, without Jen and her limitless love and encouragement, my adventures in Mississippi would have been neither as memorable nor as fulfilling. I am grateful for you all.

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CHAPTER I

INTRODUCTION

Study background

Channel catfish (*Ictalurus punctatus*) tolerate a wide range of water temperatures because they evolved in a temperate-climate region spanning from Mexico to the Great Lakes in the United States (Tavares-Dias and Moraes 2007). Although considerable research has been conducted on temperature effects on channel catfish, effects of high temperatures remain poorly understood. This lack of information on thermal biology is surprising considering channel catfish are significant to natural ecosystems (Jackson 2004a) and commercial aquaculture and water temperature is known to affect every aspect of fish physiology. In 2009, commercial channel catfish production was valued at 373 million dollars (NASS 2010) and constituted the largest section of domestic aquaculture. Paired with the importance of the fish in commercial, recreational, and artisanal fisheries, channel catfish are invaluable commodities to the fisheries community (Jackson 2004b).

Like other exothermic teleosts, ambient water temperature dictates metabolic responses in channel catfish (Brandt 1993). Water temperature also affects important functions such as growth, reproduction, and immune system function (Brett 1979; Le Morvan et al. 1998; Lang et al. 2003). Water temperatures in aquaculture ponds often

approach upper thermal tolerance ($\sim 37^{\circ}\text{C}$) levels for channel catfish (Allen and Strawn 1968), particularly in June-August which routinely see daily maximum values of 29°C and higher (Wax et al. 1987). Aquaculture ponds are generally shallow (< 1.5 m deep) and have a large surface area to volume ratio. As such, water temperatures closely follow air temperatures, which vary widely (Wax et al. 1987; E.L. Torrans, USDA-ARS, personal communication). These daily fluctuations prevent stable thermal stratification and offer little thermal refuge for catfish (Boyd 2000).

Climate change may exacerbate temperature effects on catfish aquaculture. Predictions are for a $2\text{-}3^{\circ}\text{C}$ increase in water temperature in the next century (Murphy 1995; Johnston and Ball 1997). These temperature increases may impact growth patterns in pond-raised channel catfish as well as growth and distributional patterns in natural environments (McCauley and Beitinger 1992; Ficke et al. 2007). Channel catfish have evolved in a temperate climate zone where a majority of growth occurs in the summer (Jackson 2004a). In theory, channel catfish growth rates in their central-northern distribution could increase from warming temperatures. However, in southern regions where catfish aquaculture is mainly practiced, fish may be subjected to increased thermal stress resulting in suboptimal or near-lethal conditions.

Two experimental approaches have been used to understand high temperature tolerances on channel catfish. These include the critical thermal maximum method (Cowles and Bogert 1944) and the incipient upper lethal temperature test (Hart 1952). The critical thermal maximum method involves acclimating fish to several temperatures and subsequently raising water temperature by $0.1\text{-}1^{\circ}\text{C}$ per minute until equilibrium loss occurs (Bonin et al. 1981). One study on fingerling channel catfish showed the range of

overall thermal tolerance in the critical thermal maximum method to be 38-42°C (Bennett et al. 1998). On the other hand, the incipient upper lethal temperature test involves acclimating a fish to a specific temperature and then subsequent immersion into either a warmer or cooler temperature and observing time-to-death (Otto 1974). This approach is similar to lethal dose toxicology studies. When acclimated to warm water above 30°C, the incipient upper lethal temperature value is around 37°C for fingerling catfish (Allen and Strawn 1968).

Effects of excess heat on growth and feeding

Whereas laboratory tests have generated valuable information on the effects of rapid, short-term temperature increases, they have limited applicability to fish in the wild and on commercial fish farms where temperatures cycle. In natural settings and in aquaculture ponds, temperatures increase more slowly than in most laboratory tests and fish may be exposed to a relatively narrow range of temperature for extended periods. Understanding of temperature effects under these exposure regimes remains limited. Further, growth rate, feeding rate, and feed conversion efficiency have important implications for production costs in aquaculture and have not been well studied at high temperatures.

Studies that have evaluated the effect of temperature on channel catfish feeding have focused primarily on cool-water feeding rather than high-temperature feeding (Dunham and Smitherman 1981; Tackett et al. 1987; Burtle and Newton 1993). Studies of actual feed intake at high temperatures remain essentially unidentified, although modeling analyses by Cacho et al. (1991) suggested feed intake peaks in late summer.

Farming trends suggest less food allowance during the warmest conditions (Lovell 1989). However, this is not based on the effects of water temperature on food consumption but rather on maintaining adequate dissolved oxygen and other water quality parameters (Tucker, et al. 1979; Robinson et al. 2004). Generally, as temperatures increase fish consume more oxygen, oxygen is less soluble in water, and total ammonia nitrogen concentrations spike due to degradation of uneaten feed and fish waste. The hypothesis that catfish feed, grow, and convert feed less efficiently at a high temperature range (31-35°C) compared to lower temperature ranges (23-27°C, 27-31°C) is investigated in Chapter II.

Influence of excess heat on metabolism and endurance

Increasing temperatures increase the rate of biochemical processes. As oxygen fuels these processes, oxygen supply is a limiting factor in overall metabolic capacity (Neill and Bryan 1991). Channel catfish metabolism has been well-studied (Moss and Scott 1964; Gerald and Cech 1970; Andrews and Matsuda 1975), however the relationships among oxygen consumption and high seasonal temperatures, growth, and feeding have not been elucidated. Understanding oxygen consumption in unfavorable environments, such as high temperatures, may help to explain growth and feeding results. Further, temperature effects and metabolism are linked to activity rates (Fry 1971; Beamish 1978).

Currents typify many of the habitats occupied by channel catfish. In the wild, catfish occupy riverine habitats which may require continuous or prolonged swimming. Similarly, in catfish ponds, current is generated by large paddle-wheels that move water

for aeration purposes. Paddle-wheel aerators are commonly used to supplement reduced dissolved oxygen concentrations at night when photosynthesis by phytoplankton ceases (Torrans 2008). Catfish orient to aerator currents that carry oxygenated water (Beecham et al. 2007). As currents vary and dissipate depending on location, velocities may often be as high as 85 cm/second within one meter of the paddle-wheel (Beecham et al. 2009).

Diminished dissolved oxygen occurs in hot weather and is exacerbated by the degradation of uneaten feed, phytoplankton blooms, and increased metabolic demands in catfish. Swimming activity by catfish orienting to aerator currents creates additional metabolic demands which further increase the oxygen debt. These combined factors have the capacity to reduce metabolic scope, thereby preventing optimum metabolic efficiency and negatively affecting growth (Neill and Bryan 1991). However, these are the conditions that fish are commonly subjected to on commercial farms and the overall impact on fish growth, health, and survival is not quantified. An investigation of swimming endurance, active, and resting metabolic rates of three elevated temperature levels (27, 31, and 35°C) is presented in Chapter III.

Purpose of study

The overall objective of these studies was to measure the effect of high seasonal temperatures on feeding, growth, metabolic rate and swimming endurance in channel catfish, with application to summer water temperatures of farm ponds and increased summertime water temperatures resulting from changing climatic conditions. Climate change is expected to increase average water temperatures 2-3°C in the next century (Murphy 1995; Johnston and Ball 1997), which may create additional complications in

already-stressed fish during summers in Southern states. Test results should help to define thermal physiological constraints of an important food crop and may help to suggest new strategies for summertime feeding regimes.

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CHAPTER II

INFLUENCES OF CYCLIC, HIGH TEMPERATURES ON JUVENILE CHANNEL CATFISH GROWTH AND FEEDING

Introduction

Channel catfish (*Ictalurus punctatus*) tolerate a wide range of water temperatures because they evolved in a temperate climate region which spans from Mexico to the Great Lakes in the United States (Tavares-Dias and Moraes 2007). Within that region, water temperatures vary from 0°C to over 30°C. Like other exothermic teleosts, channel catfish metabolism is affected by ambient water temperature, which directly influences bioenergetic responses in many body systems (Brandt 1993). Water temperature affects important functions such as growth, reproduction (Lang et al. 2003), and immune system function (Le Morvan et al. 1998).

Water temperatures in aquaculture ponds currently approach upper thermal tolerance (~ 37°C) levels for channel catfish (Allen and Strawn 1968), particularly in June-August which routinely see daily maximum values of 29°C and higher (Wax et al. 1987). Aquaculture ponds are generally shallow (< 1.5 m deep) and have a large surface area to volume ratio. As such, water temperatures closely follow air temperatures, which vary widely (Wax et al. 1987). These daily fluctuations prevent stable thermal stratification and offer little thermal refuge for catfish (Boyd 2000).

Water temperatures approach upper temperature tolerance under normal seasonal conditions in Mississippi and other traditional catfish aquaculture regions. Climate change may further impact growth patterns in pond-raised channel catfish as well as growth and distributional patterns in natural catfish fisheries (McCauley and Beitingger 1992; Ficke et al. 2007). Channel catfish evolved in a temperate climate zone where a majority of growth occurs in summer and could benefit from warming temperatures in natural environments where growth is limited by cool temperatures.

Thermal tolerance tests such as critical thermal maximum and incipient upper-lethal temperature have generated valuable information on effects of rapid, short-term temperature increases in channel catfish (Allen and Strawn 1968; Bennett et al. 1998), yet have limited applicability to fish in the wild and on commercial fish farms. In natural settings and on farms, temperatures increase at slower rates and may be maintained for extended periods in comparison to methods used to determine critical thermal maximum and incipient upper lethal temperature tests (Randolph and Clemens 1976). Thus, the understanding of temperature effects under these types of exposure regimes remains limited. Growth rate, feeding rates, and feed conversion efficiency are significant factors to production costs and also have not been well studied for high temperatures.

Studies that have evaluated the effect of temperature on feeding have focused primarily on cool-water feeding rather than high temperature feeding (Dunham and Smitherman 1981; Tackett et al. 1987; Burtle and Newton 1993). Few studies of feed consumption, growth, and feed conversion efficiency have been conducted at high temperatures. Farmers feed reduced rations during the warmest conditions (Lovell 1989). However, this is largely dependent on the farmer and this practice is not typically based

on food consumption rate but rather on maintaining proper dissolved oxygen and other water quality parameters (Robinson et al. 2004).

Therefore, the objectives of this study are to compare growth and feeding of juvenile channel catfish subjected to three treatments of cyclic, elevated temperature ranges representative of summer conditions in the Mississippi Delta: 23-27, 27-31, and 31-35°C (E.L. Torrans, USDA-ARS, personal communication). The hypothesis that catfish grow, feed, and convert feed less efficiently at a high temperature range (31-35°C) compared to lower temperature ranges (23-27°C, 27-31°C) is investigated in this research.

Methods

Fish source and pre-experimental acclimation

Fingerling channel catfish were purchased from a commercial supplier (L & S Fish Farms, Leland, MS) and transferred to two raceways (3 x 0.9 x 0.6 m) at the South Farm Aquaculture Facility, Mississippi State University, USA. Raceways were supplied with non-chlorinated, flow-through aerated well water at 26°C. For determining appropriate food quantities, a subsample of 30 fingerling catfish was measured and weighed individually to obtain initial total length (10 ± 2 cm) and wet weight (17 ± 3 g). Juvenile catfish of this size are typical of catfish moved from fry/fingerling ponds to grow-out ponds in an aquaculture production setting (Bader and Grizzle 1992; Bonn and Follis 1967). Fish were fed a pelleted, 40% protein diet (Rangen Inc., Buhl, ID) at 6% body weight (BW) per day based upon a protocol derived from Dupree (1984), with most

food (5% BW) fed in the morning at 0900 and an additional 1% BW in the afternoon at 1630. Catfish were maintained in raceways for 5 weeks prior to experiments.

System design and thermal regimes

Three cyclical water temperature regimes were tested: 23-27°C, 27-31°C, and 31-35°C. These regimes were based on summer water temperature data recorded in six, 0.25-ha experimental ponds at the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS. A buoy (Royce Technologies, Charlotte, NC) sampled and recorded the temperature of mid-depth pond water at 60 cm below the surface every 6 minutes from May-November 2009.

For the 27-31°C and 31-35°C treatments, well-water was supplied to a 7,500-L preheating reservoir where water temperature was increased to 27°C by a submersible, titanium 3,000-watt heater (Clepeco Inc., Homestead, FL). Inflow to this reservoir was maintained by two stock tank float valves (Rubbermaid Inc., Atlanta, GA). The preheated water was then pumped to two elevated 474-L head tanks (Rubbermaid Inc., Atlanta, GA) where it was warmed to treatment temperatures using 1,500-watt heaters (Clepeco Inc., Homestead, FL). For the 23-27°C treatment, the incoming well water was chilled in a separate 474-L reservoir using a chiller (Frigid Units Inc., Toledo, OH) that was capable of reducing the temperature to 23°C. Because the well-water equilibrated with the ambient air temperature, no additional heating was needed to raise the temperature to 27°C.

Cycling temperature regimes were used to duplicate actual pond conditions. Temperature cycles in production ponds cycle approximately 4°C per day (Wax et al.

1987). To obtain a similar temperature cycle, heaters were controlled by a series of timers. Temperatures were increased by additional heaters which were programmed to start at 1300 for the 27-31°C treatment and at 0900 and 1300 for the 31-35°C treatment to correspond with natural daily temperature increases (Figure 2.1). All head tanks were covered with rigid foam insulation (Dow Inc., Midland, MI) as well as wrapped with reflective foil insulation (Reflectix Inc., Markleville, IN) to maintain treatment temperatures.

Water from head tanks was distributed via flexible polyvinyl chloride (PVC) hosing to rigid PVC pipes which gravity-fed the temperature-treated water to a lower platform containing experimental tanks which housed the catfish. Each of the three temperature treatment reservoirs fed six replicate 114-L rectangular 30 x 76 x 48 cm experimental tanks totaling 18 tanks in a complete randomized design. Tanks were insulated with reflective foil insulation (Reflectix Inc., Markleville, IN) applied with silicone to the exterior of the tank, and covered with egg crate screening to prevent escape (Figure 2.2). Incoming water flow rates were set to maintain a 70-minute water turnover rate with no recirculation, providing optimum water quality.

Growth experiment

Fingerling channel catfish were randomly selected from holding tanks, lightly anesthetized with 150 mg/L tricaine methanesulfonate (MS-222) (Argent Chemical Laboratories Inc., Redmond, WA), individually weighed to the nearest ± 0.1 g, and measured for standard length and total length to the nearest ± 1 mm. Twenty fish were placed in each tank, with six tanks per treatment totaling 360 fish, and allowed to

acclimatize for 24 hours before feeding commenced. To determine initial dry weights of fish, 12 catfish were randomly selected from the holding tanks and euthanized with 500 mg/L of MS-222. These fish were measured for initial wet weights, standard lengths, and total lengths as described above. Fish were dried at 80°C in an oven (Fisher Scientific Inc., Pittsburgh, PA) for 10 days, when a constant dry weight was measured. Samples were then cooled in a dehumidifying chamber prior to determining final dry weights.

During feeding, incoming water was turned off prior to the introduction of food to prevent accidental loss of feed through the outflow pipe of each tank. The catfish were fed to satiation over a 20-minute period using a formulated 2.5-mm pelleted 40% protein trout production diet (Rangen Inc., Buhl, ID). If all food was consumed, an additional 1 g of feed was added, and fish were given an additional 10 minutes to consume the food. This was repeated until there was a substantial amount (> 50 pellets) of uneaten food remaining. Upon cessation of feeding, uneaten pellets were netted out and counted. The weight of an individual pellet was determined prior to the experiment by conducting an average of the weight of 500 pellets. By counting uneaten pellets and multiplying by the weight per pellet, the amount of feed consumed to the nearest ± 0.01 g was concluded. This protocol was repeated daily for the entire 8-week experiment for each tank, except for a 48-hour starvation period immediately prior to the 4-week and 8-week weigh-ins. Any mortalities were recorded prior to feeding each day and removed.

After the 8-week period, final wet weights (nearest ± 0.01 g), standard lengths (nearest ± 1 mm), and total lengths (nearest ± 1 mm) were recorded. For dry weights, a subsample of two randomly selected fish per tank were removed and dried as previously

described. Feed conversion rate (FCR) was calculated according to Davis and Stickney (1978) where:

$$FCR = (\text{g fed} / \text{g weight gained}) \quad (2.1)$$

Specific growth rate (SGR) was calculated from Peterson and Small (2006) where:

$$SGR = \{[\log_e(\text{final weight}) - \log_e(\text{initial weight})]/(\text{number of days})\} \times 100 \quad (2.2)$$

Weight adjusted daily feed consumption was calculated from Silverstein et al. (2000) where:

$$\text{Total weight of feed consumed over the 8-week study (g)} \times (\text{start fish weight g} + \text{end fish weight g}/2)^{-1} \times \text{number of days}^{-1}. \quad (2.3)$$

Water quality

Water quality was measured three times per week (Table 2.1). Total ammonia-nitrogen (TAN) and nitrite (NO_2^-) were measured using a colorimetric reader (Hach DR550, Hach Company, Loveland, CO). Water pH was measured using a glass electrode and pH meter (pH10N, YSI Inc., Yellow Springs, OH) and dissolved oxygen (DO) concentrations and temperature were measured using a DO meter and polarographic probe (Y55, YSI Inc., Yellow Springs, OH). Daily changes in temperature were measured at 15-minute intervals by temperature data loggers (Onset Corporation, Bourne,

MA) throughout the experiment. Un-ionized ammonia (NH₃) was calculated using TAN, temperature, and pH.

Table 2.1 Water quality variables measured for an 8-week growth experiment. Values are listed as means \pm SE, $n = 24$. Data were taken three times weekly from August-October 2010, Mississippi State, MS.

Temp (°C)	TAN ¹ (mg/L)	NH ₃ (mg/L)	Nitrite (mg/L)	pH	DO ² (mg/L)	Saturation (avg. %)
23-27	0.10 \pm 0.02	0.01 \pm 0.00	0.01 \pm 0.01	8.0 \pm 0.12	5.95 \pm 0.15	72.2
27-31	0.11 \pm 0.03	0.01 \pm 0.00	0.01 \pm 0.01	8.0 \pm 0.11	5.94 \pm 0.10	77.5
31-35	0.11 \pm 0.05	0.01 \pm 0.01	0.01 \pm 0.01	8.1 \pm 0.13	6.02 \pm 0.13	83.6

¹Total ammonia-nitrogen

²Dissolved oxygen

Activity

To determine if elevated temperature levels had an effect on fish movement, swimming activity was passively measured using a high definition video camera (Sanyo Xacti VPC-GH2, Sanyo Electric Co., Ltd., Osaka, Japan). Activity measurements were recorded several days past the 4-week midterm weighing. Fish movements in one tank at a time were recorded for a 20-minute interval using a 111-cm tall mount which secured and angled the camera down over the tank. Movements in the first 15 minutes were not quantified to allow for acclimation to the camera mount. Movement data was quantified from the final 5 minutes on a computer monitor by overlaying a 5 x 5 cm grid in actual tank dimensions. Tanks and catfish were randomly selected, and two catfish per tank were observed for 30 seconds within the 5-minute recording period. A tally was recorded

of how many grids the head of each fish crossed during that period. No vertical movement was accounted for in these observations. Eight fish per treatment were measured for activity totaling 24 fish.

Statistical analysis

Data are expressed as means \pm standard errors (SE). Wet weight ($P = 0.001$) and activity data ($P = 0.015$) were not normally distributed (Shapiro-Wilk) so Kruskal-Wallis nonparametric analysis of variance (ANOVA) was used to determine differences for these variables. Feed conversion rate, specific growth rate, feed consumption, standard length, and total length were normally distributed as determined by Shapiro-Wilk tests and further analyzed by one-way ANOVA or *t*-test in the case of food consumption. Statistically significant values were assessed using a Tukey's *post-hoc* Honestly Significant Difference Test (JMP version 9.0.2; SAS Institute, Cary, North Carolina). In all cases $\alpha = 0.05$.

Results

Growth

Fish grew in all treatments, with increases in standard length (Figure 2.3), total length (Figure 2.4), and wet weight (Figure 2.5). However, fish in the 27-31°C treatment had greater standard lengths and wet weights in comparison to fish in 23-27°C and 31-35°C treatments at 4-week and 8-week weigh-ins. A blockage in a water supply line occurred after the 4-week measurement which prevented adequate water turnover for two

of the 31-35°C treatment tanks and killed most of the fish in these tanks by the following morning. Therefore, these tanks were not included in analyses (including survival rates) after week 8.

After 8 weeks, the fish in the 23-27°C treatment had a final mean \pm SE standard length of 132.8 ± 16.5 cm, total length of 161.8 ± 18.6 cm, and wet weight of 37.1 ± 14.6 g. This indicated growth increases of 18.3% for standard length, 22.6% for total length, and 58.5% for wet weight. Catfish in the 27-31°C treatment obtained a final mean \pm SE standard length of 147.8 ± 19.2 cm, total length of 184.0 ± 23.7 cm, and wet weight of 56.7 ± 24.4 g. This indicated growth increases 25.4% for standard length, 24.2% for total length, and 69.0% for wet weight. Catfish in the 31-35°C treatment had a final mean \pm SE standard length of 135.4 ± 18.9 cm, total length of 168.3 ± 24.1 cm, and wet weight of 41.8 ± 19.0 g. This indicated growth increases of 18.8% for standard length, 17.4% for total length, and 56.9% for wet weight.

Feed conversion rate

Feed conversion efficiency (mean \pm SE) for fish in the 23-27°C treatment was 1.28 ± 0.05 . Catfish in the 27-31°C treatment had a feed conversion efficiency of 1.07 ± 0.05 , whereas catfish in the 31-35°C treatment had 1.44 ± 0.06 (Figure 2.6). Fish in the 27-31°C treatment differed significantly from the other temperature treatments ($P = 0.003$) (one-way ANOVA, Tukey-Kramer's HSD test.)

Specific growth rate

Specific growth rate (mean \pm SE) for fish in the 23-27°C treatment was 1.57 ± 0.06 . Catfish in the 27-31°C treatment had a specific growth rate of 2.05 ± 0.06 , whereas catfish in the 31-35°C treatment had 1.55 ± 0.08 (Figure 2.7). Fish in the 27-31°C treatment differed significantly from the other temperature treatments (one-way ANOVA, Tukey-Kramer's HSD).

Feed consumption

Daily feed consumption as a percentage of body weight (mean \pm SE) was found to be $2.34 \pm 0.11\%$ for catfish held in the 23-27°C treatment, whereas catfish held at 27-31°C consumed $2.78 \pm 0.22\%$ and catfish at 31-35°C consumed $2.36 \pm 0.18\%$ (Figure 2.8). There was a significant difference in these consumption rates showing the 27-31°C treatment consumed the most feed per day (one-way ANOVA, Tukey-Kramer's HSD, $P = 0.003$).

Activity

Activity (number of grid boxes crossed in 30 seconds, displayed as mean \pm SE) for catfish in the 23-27°C treatment was 24.38 ± 2.91 . Catfish in the 27-31°C treatment level crossed 25.25 ± 2.91 boxes, whereas catfish in the 31-35°C treatment crossed 36.38 ± 2.91 boxes (Figure 2.9). There was a significant difference in activity ($P = 0.022$), with the greatest activity in fish in the 31-35°C treatment, whereas catfish in the 23-27 and 27-31°C treatments were not different from one another (Kruskal-Wallis, Tukey-Kramer HSD).

Discussion

The findings of this study indicate that juvenile channel catfish are well adapted for growth at temperatures of 27-31°C which is supported by findings in Buentello et al. (2000) and Hariyadi et al. (1994). Fish held in this temperature range had the best feeding and growth. Andrews and Stickney (1972) also found that channel catfish fingerlings grew and converted feed best at 30°C. Shrable et al. (1969) observed the greatest rate of digestion at 29°C. With an increase in temperature to 31-35°C, growth and feed consumption decreased with a corresponding increase in activity. Andrews and Stickney (1972) also found that growth and feed conversion of channel catfish fingerlings declined at high (34°C) sustained temperatures.

Survival decreased with increasing temperature (Figure 2.10). Although the 31-35°C treatment approached the limits of thermal tolerance, survival was high likely due to otherwise good water quality. However, catfish in this temperature treatment developed minor skin lesions and fin degradation as the experiment progressed although the nature and cause of these lesions were not determined by formal diagnostic procedures. Temperatures outside optimum range strongly affect immunocompetency and therefore may be an underlying cause of the observed lesions (Hargreaves and Tomasso 2003; Bowden et al. 2007).

Catfish from the 31-35°C treatment had greater activity than catfish from the other two temperature treatments. Increased movements of fish from the high temperature treatment may be due to discomfort, and may indicate that fish were stressed at this temperature range. Fry (1971) described this behavior, with Otto and Rice (1977) observing similar activity relationships in thermally stressed freshwater sculpin (*Cottus*

cognatus gracilis), as well as Cherry et al. (1976) in mosquitofish (*Gambusia affinis*).

Other observations suggesting thermal stress at this temperature range were decreased survival, poor appearance, and poor feed conversion ratio and specific growth rate.

Feed conversion ratio was most efficient in fish in the 27-31°C treatment group, although values were good (1.07-1.44) in all treatments indicating that catfish converted feed to weight efficiently. Specific growth rate values follow the same relationship as feed conversion rate, with greater growth rates in the 27-31°C treatment compared to the 23-27°C and 31-35°C treatments. Therefore, sustained elevated temperatures have a negative effect on specific growth rate. Furthermore, catfish in sustained elevated temperatures consumed less feed as a percentage of body weight. Relationships between specific growth rate and feed conversion ratio are generally correlated, with the results in this study showing that poor feed conversion rates contribute to reduced growth rates. Several factors determine this relationship, namely fish size, temperature, and ration (Nordgarden 2003; Lien 2007). In sum, the catfish held at 27-31°C consumed the most feed, converted it most efficiently, and grew fastest.

The coolest (23-27°C) and warmest (31-35°C) temperature treatments had reduced growth rates indicating non-optimal conditions for growth. These results agree with the findings of previous studies, which found reduced growth in juvenile channel catfish at temperatures < 23°C (Lovell 1974) and > 34°C (Andrews and Stickney 1972). The results of this study indicate that good growth is possible at water temperatures typical of summertime in Mississippi. However, growth decreases at water temperatures approaching the upper thermal tolerance range for channel catfish fingerlings at 37-42°C (Bennett et al. 1998; Currie et al. 1998). Decreases in food consumption, feed conversion

ratio, and growth at unfavorably warm or cool temperatures are also documented in other warmwater fish species such as brown bullhead (*Ictalurus nebulosus*) (Keast 1985), bluegill (*Lepomis macrochirus*) (Beitinger and Magnuson 1979), and white crappie (*Pomoxis annularis*) (Hayward and Arnold 1996).

These feeding, growth, and activity assessments illustrate several important physiological facets of juvenile channel catfish in high temperature environments. Fish in the 27-31°C treatment group had the best performance in feed conversion efficiency and feed intake, resulting in rapidly growing fingerlings. At cooler temperatures and, more importantly, at warmer temperatures, growth and feed conversion were not as good in channel catfish as illustrated by Fry (1971). Reduced growth and feeding at high temperatures have been found in similar studies on other temperate fish species, such as rainbow trout (Myrick and Cech 2000), brown trout (Elliot 1975), and common carp (Hwang and Lin 2002). Poor growth is exacerbated by greater activity rates, which contributed to lesser weight gains. Fish in the lower temperature treatments did not increase energy use through active movement.

McCauley and Beitinger (1992) suggested that climate change may expand opportunities for commercial catfish culture in more northern locations. However, based on the results of this study, it may come with the cost of reduced production efficiency in the southeast. Further, according to the findings of decreased feed conversion rate, farmers may want to adopt different feeding strategies similar to reduced winter rations (Lovell and Sirikul 1974) on exceptionally warm summer days if feeding *ad libitum*. Models from Cacho et al. (1991) suggest that reducing feeding rates is a strategy to

improve feed conversion efficiency during episodes of high water temperature. This may also help to alleviate impaired water quality caused by uneaten feed.

This study shows a decrease in growth and feeding performance for fingerling channel catfish exposed to elevated water temperatures. Future research should focus on temperature effects on market-sized (500-1,000 g) channel catfish. As fish increase in size, temperature tolerance narrows (Brett 1979). Additionally, grow-out ponds differ from fingerling ponds in that annual drainages do not often occur, and the increased biomass and feeding rates produce more phytoplankton increasing oxygen debts, which create additional stress in larger catfish in high temperatures. A separate experiment examining hybrid catfish (*Ictalurus punctatus* \times *I. furcatus*) would also be beneficial due to its increasing importance in aquaculture production. Future studies should also seek to incorporate additional stressors that catfish typically endure in earthen ponds, such as nitrogenous wastes and, in particular, fluctuating dissolved oxygen levels because oxygen is a limiting factor of fish metabolism.

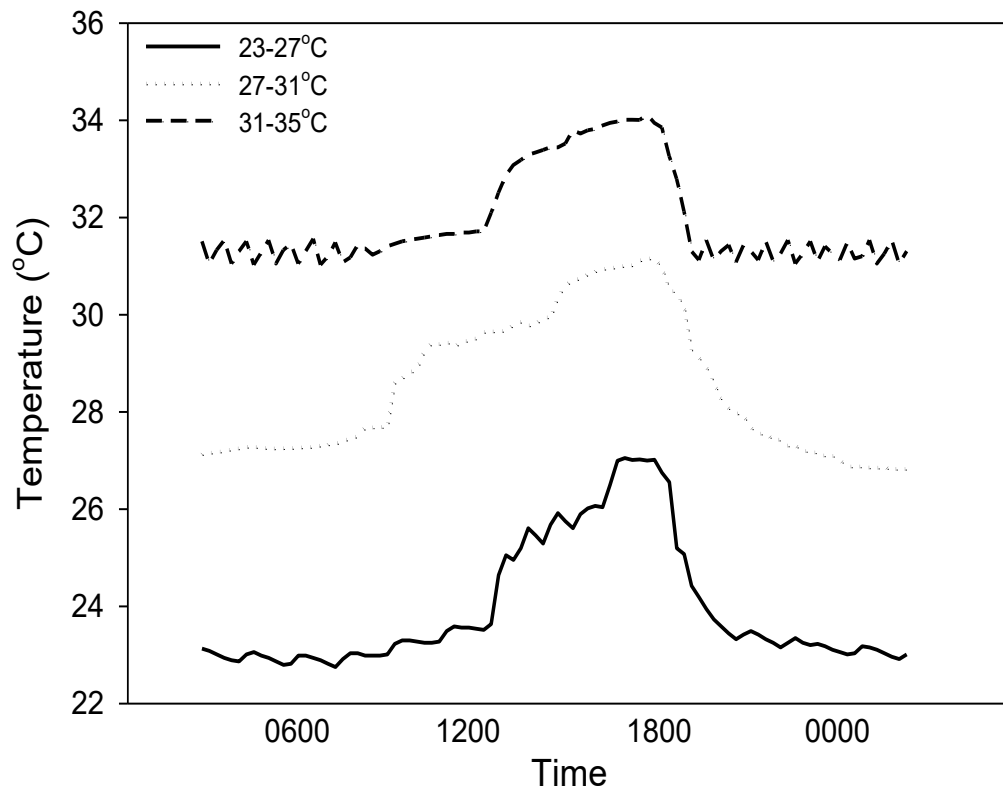


Figure 2.1 Representative 24-hour temperature cycles in treatment tanks during an 8-week growth experiment reflective of summer water temperatures from the Mississippi Delta (Torrans 2010). Project occurred from August-October 2010, Mississippi State, MS.



Figure 2.2 Experimental flow-through system for testing 3 daily, cyclic water temperatures (23-27, 27-31, and 31-35°C) characteristic of Mississippi Delta summer conditions on juvenile channel catfish (*Ictalurus punctatus*) growth, feeding, and activity. Note pre-heating reservoir (left) and head tanks (rear, center) which gravity-fed water to 18 randomized research tanks housing channel catfish ($n = 20$ fish per tank). Project occurred from August-October 2010, Mississippi State, MS.

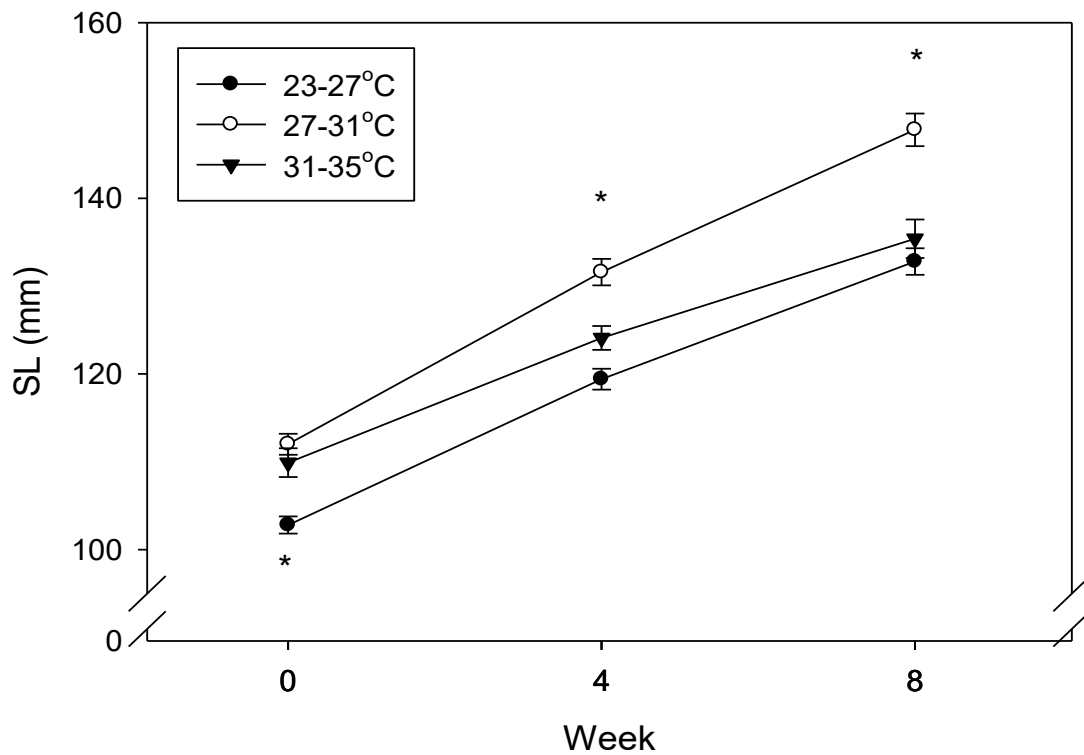


Figure 2.3 Mean (\pm SE) standard length (SL) values for juvenile channel catfish (*Ictalurus punctatus*) in an 8-week growth experiment. Asterisks indicate significant differences among treatments at each week (two-way ANOVA, Tukey-Kramer HSD, $P < 0.05$; $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

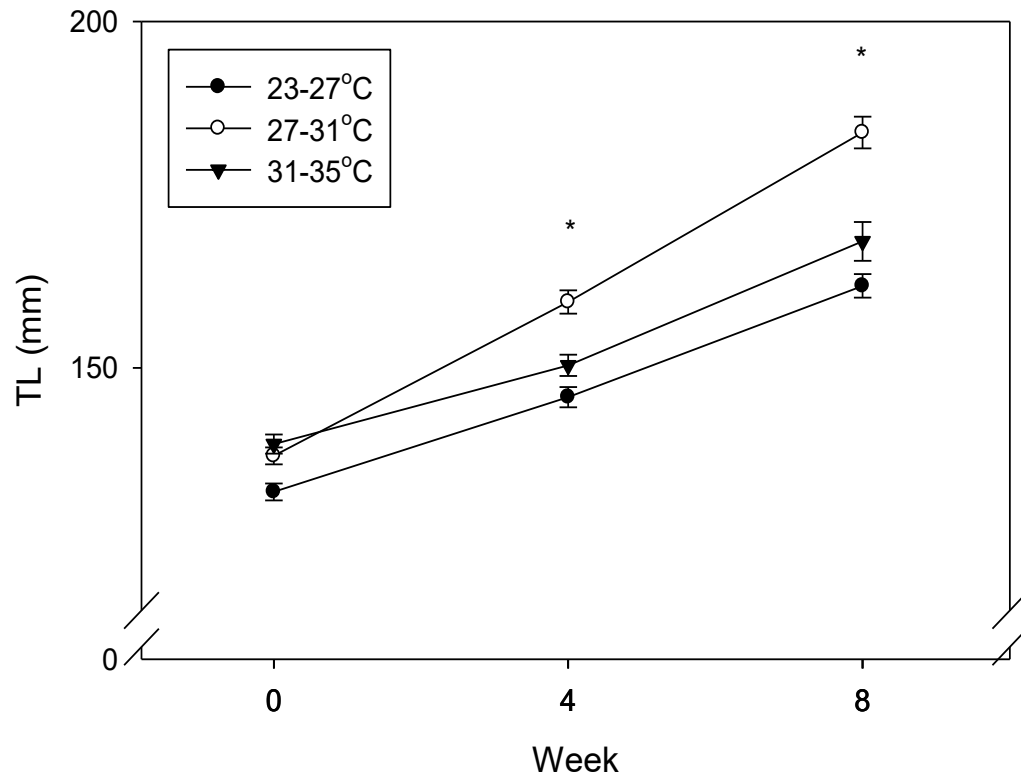


Figure 2.4 Mean (\pm SE) total length (TL) values for juvenile channel catfish (*Ictalurus punctatus*) in an 8-week growth experiment. Asterisks indicate significant differences among treatments at each week (two-way ANOVA, Tukey-Kramer HSD, $P < 0.05$; $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

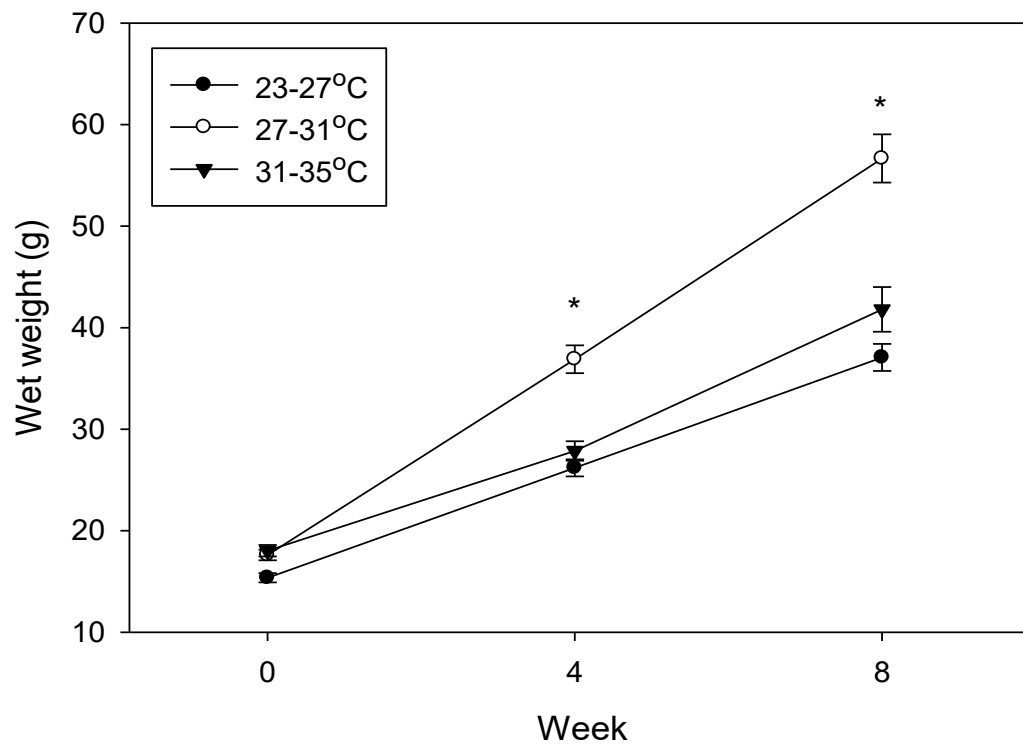


Figure 2.5 Mean (\pm SE) wet weight values for juvenile channel catfish (*Ictalurus punctatus*) in an 8-week growth experiment. Asterisks indicate significant differences among treatments at each week (two-way ANOVA, Tukey-Kramer HSD, $P < 0.05$; $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

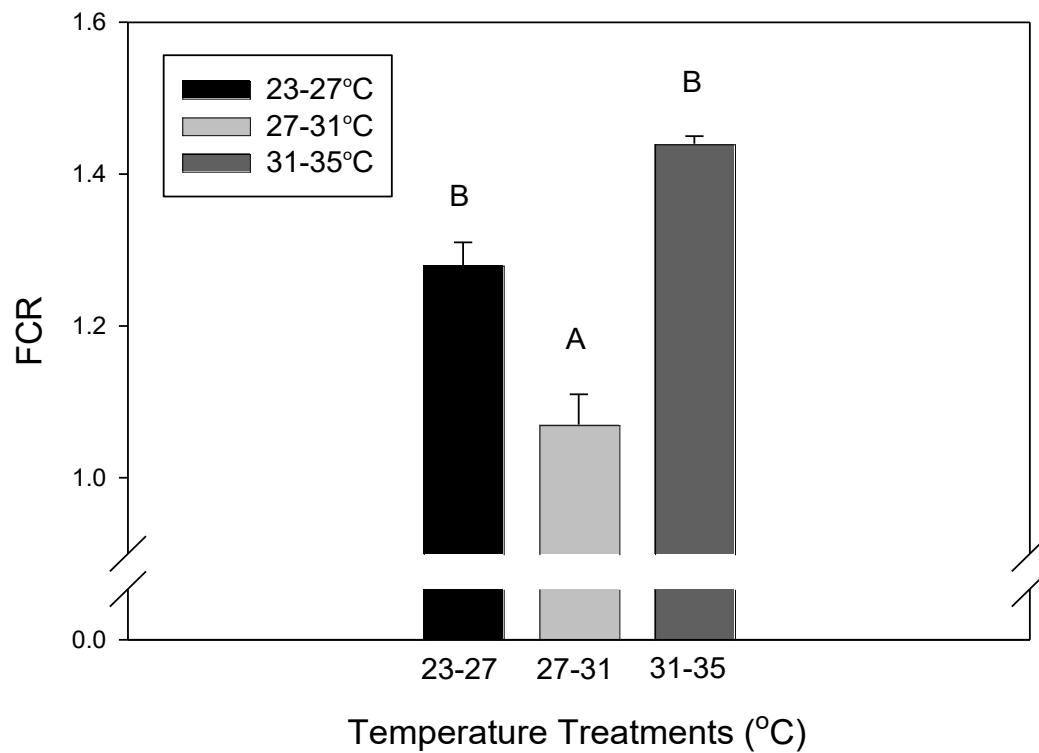


Figure 2.6 Mean (\pm SE) food conversion rate (FCR) for juvenile channel catfish (*Ictalurus punctatus*) in cyclic water temperature treatments of 23-27, 27-31, and 31-35°C after an 8-week growth experiment. Letters indicate significant differences among treatments at $P < 0.05$ (one-way ANOVA, Tukey-Kramer HSD, $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

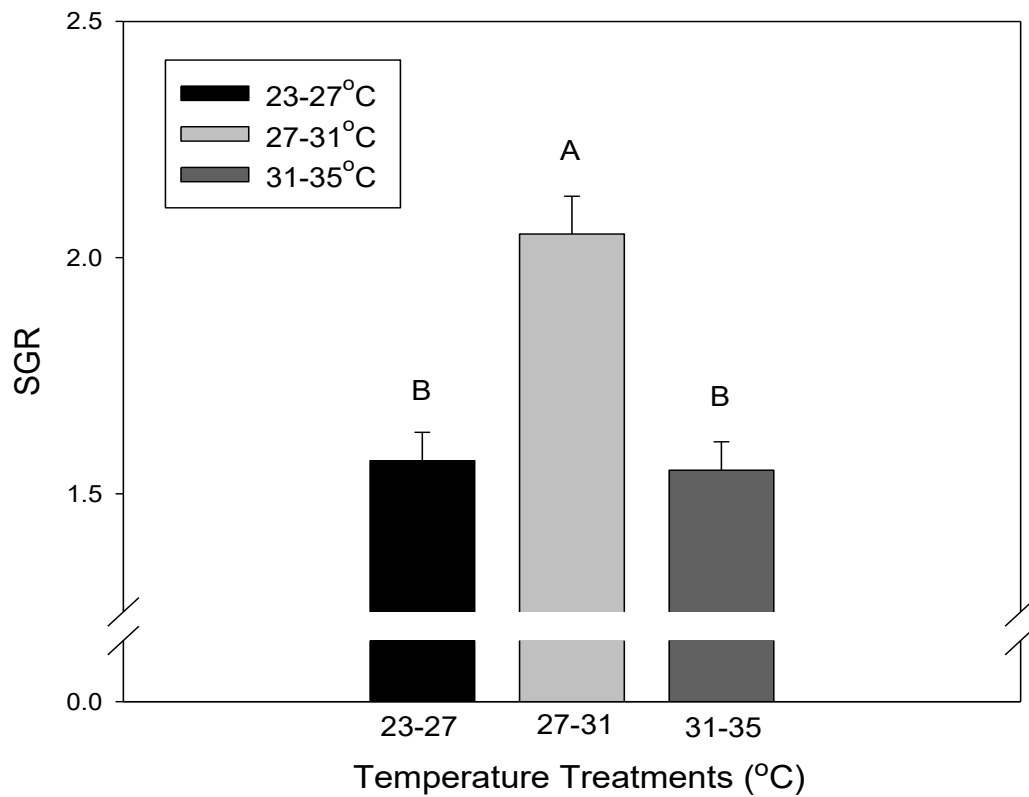


Figure 2.7 Mean (\pm SE) specific growth rate (SGR) for juvenile channel catfish (*Ictalurus punctatus*) in cyclic water temperature treatments of 23-27, 27-31, and 31-35°C after an 8-week growth experiment. Letters indicate significant differences among treatments at $P < 0.05$ (one-way ANOVA, Tukey-Kramer HSD, $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

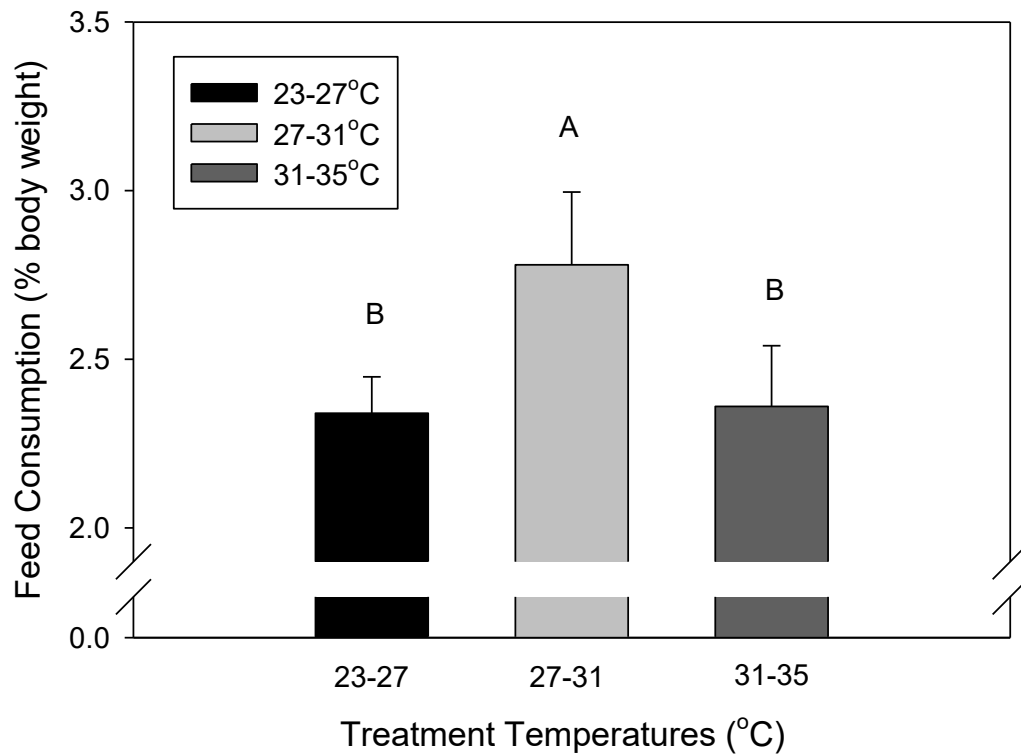


Figure 2.8 Daily food consumption expressed as a percentage of body weight for juvenile channel catfish (*Ictalurus punctatus*) in cyclic water temperature treatments of 23-27, 27-31, and 31-35°C after an 8-week growth experiment. Letters indicate significant difference in food consumption among treatments at $P < 0.05$ (one-way ANOVA, Tukey-Kramer HSD, $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

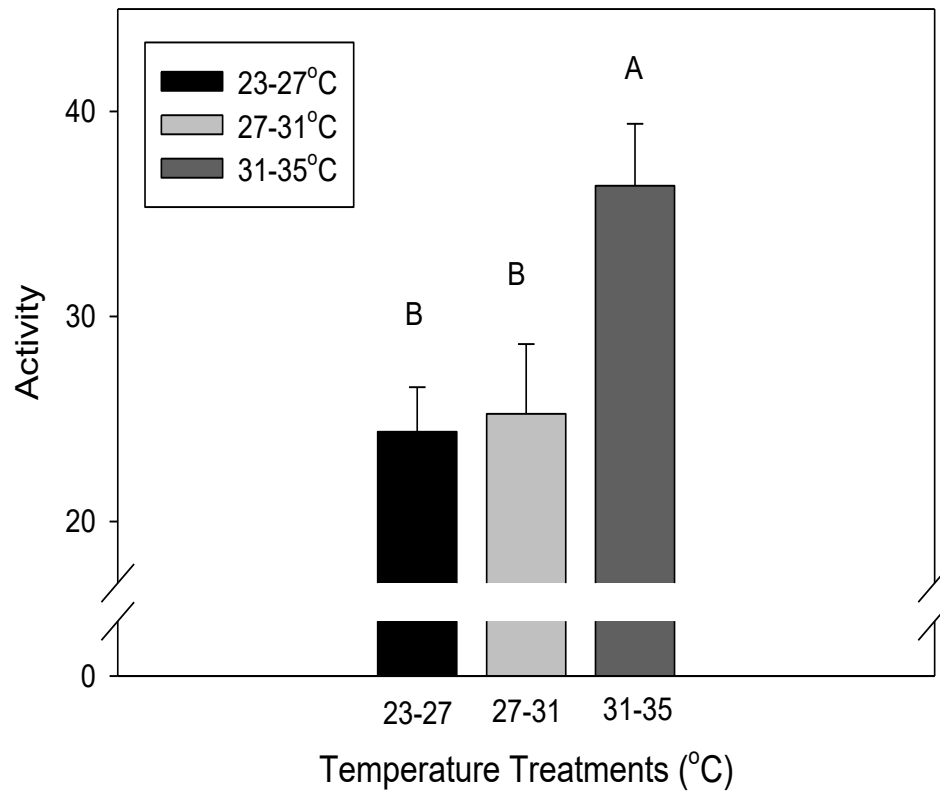


Figure 2.9 Mean (\pm SE) activity (number of grid boxes crossed in 30 seconds) for juvenile channel catfish (*Ictalurus punctatus*) in cyclic water temperature treatments of 23-27, 27-31, and 31-35°C after an 8-week growth experiment. Letters indicate significant differences among treatments at $P < 0.05$ (one-way ANOVA, Tukey-Kramer HSD, $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

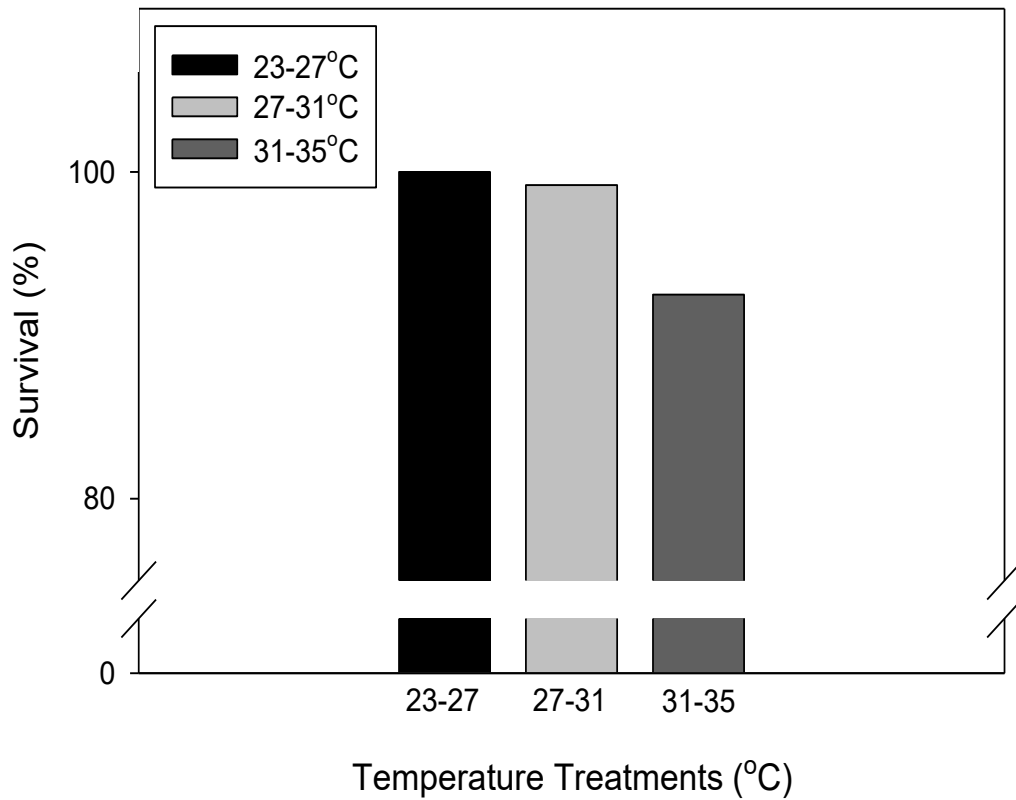


Figure 2.10 Mean survival rate for juvenile channel catfish (*Ictalurus punctatus*) in cyclic water temperature treatments of 23-27, 27-31, and 31-35°C after an 8-week growth experiment. No significant difference was found among treatments at $P < 0.05$ (one-way ANOVA, $n = 4-6$ tanks per treatment). Project occurred from August-October 2010, Mississippi State, MS.

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CHAPTER III

THE INFLUENCES OF HIGH TEMPERATURES ON AEROBIC METABOLIC RATE
AND SWIMMING ENDURANCE IN JUVENILE
CHANNEL CATFISH

Introduction

Metabolism is the sum of the biochemical reactions which produce energy exploited by an organism for maintenance and activity (Fry 1971; Brett and Groves 1979). Metabolism is strongly influenced by temperature and dissolved oxygen, where temperature has a controlling effect and oxygen has a limiting effect on metabolic rates in poikilotherms such as fishes (Clausen 1933; Fry 1971; Brett and Groves 1979; Neill and Bryan 1991). Temperature controls the rate of metabolic reactions, which provide energy for physiological functions such as swimming activity, feeding, growth, and spawning (Johnston and Dunn 1987; Houlihan et al. 2001; Hochachka and Somero 2002). Dissolved oxygen is the common reactant for biochemical reactions, and its supply can limit the rate of reactions regardless of temperature (Fry 1971; Neill and Bryan 1991). Oxygen-limited metabolism is more common at high temperatures because greater reaction rates require greater quantities of oxygen.

Many fish species (e.g., largemouth bass *Micropterus salmoides*) increase movements and feeding with increasing temperatures, and decrease these activities when

temperatures decline in preparation for winter dormancy (Cech et al. 1979).

Channel catfish (*Ictalurus punctatus*) have a similar temperature-activity relationship, and aquaculturalists adjust their management strategies accordingly (Dunham and Smitherman 1981; Robinette et al. 1982). Feed quantity and frequency are reduced during winter months in response to decreasing metabolic rates and resulting decreased feed consumption (Lovell and Sirikul 1974). As demonstrated in the previous chapter, high temperatures also reduce feed consumption. Causal factors of this phenomenon are not well understood, but quantifying metabolic rates will help to clarify underpinning relationships between temperature and bioenergetic costs.

Several levels of metabolic rates have been described in fishes, including basal, resting, routine, and active (Wieser 1985), with each level varying among species, life stage, size, and location (Evans 1984; Lucas et al. 1993; Kieffer et al. 1996). Measuring these levels in fishes typically involves observation of oxygen consumption, which is measured as the decline in water oxygen content over a period of time (Cech 1990). Determining the difference between resting and active metabolic rates produces the metabolic scope for activity (Fry 1947). Scope for activity is useful for estimating the influence of environmental parameters such as temperature and dissolved oxygen concentration on physiological performance capabilities (i.e., growth and swimming) (Fry 1971; Neill and Bryan 1991).

Measurements of swimming activity and exercise are important for understanding physiological capabilities and resulting ecological influences such as the evaluation of fitness in terms of offspring/generational success (Plaut 2001), foraging performance (Webb 1984), predator avoidance capability (Domenici 2001), and certainly have

implications for migrational species (Castro-Santos 2004). Temperature influences metabolic efficiency, and therefore has an effect on swimming performance (Lee et al. 2003).

Swimming endurance is a measure of sustained swimming capability, typically for a 200-minute time period (Beamish 1978) and is particularly applicable to channel catfish in pond culture systems. Large paddlewheel aerators generate currents in aquaculture ponds to increase water oxygen concentrations. Although channel catfish evolved as riverine fishes (McMahon and Terrell 1982), they may become fatigued under forced swimming conditions created by the sustained water currents. Their swimming capabilities may be decreased due to a lack of exercise from the otherwise static culture environments of ponds and may be further affected by high temperatures.

Therefore, the objectives of this study were to measure channel catfish resting and active aerobic metabolic rates at three high temperatures (27, 31, and 35°C) and to calculate the metabolic scope for activity. These temperatures are representative of summer water conditions in the Mississippi Delta, the primary location of channel catfish aquaculture production in the US (E.L. Torrans, USDA-ARS, personal communication). Because even brief exposures of sub-optimal temperatures can have large effects on physiological performance (Logue et al. 1995), it is important to understand thermal effects under actual pond conditions. Similar to previous studies on the role of increasing temperature on metabolic rates of poikilotherms (Johnston and Dunn 1987; Schurmann and Steffensen 1997), the hypothesis that fish acclimated to 35°C will have the greatest metabolic rates for active and resting levels and exhibit the least swimming endurance will be examined in this study.

Methods

Fish source and pre-experimental acclimation

Fingerling-sized channel catfish were purchased from a commercial supplier (L & S Fish Farms, Leland, MS) and transferred to two raceways (3 x 0.9 x 0.6 m) at the South Farm Aquaculture Facility, Mississippi State University, USA. Raceways were supplied with non-chlorinated, flow-through aerated well water at 26°C and held the catfish for 1 year, reaching stocker size (100-250 g) at the start of this study. While held in the raceways, catfish were fed a formulated 36% protein fingerling feed (Land O Lakes Inc, St. Paul, MN) at 5% body weight daily. Immediately prior to experiments, a sub-sample ($n = 6$) of fish were randomly sampled from the raceway and were weighed for initial weight (nearest 0.1 g) and measured for total length (TL, nearest mm). Mean \pm standard error (SE) wet weight was 165.1 ± 16.4 g and TL was 276 ± 14 mm (Table 3.2).

System design, thermal treatments, and feeding

Seventy-five catfish were randomly distributed among three circular, insulated holding tanks (7,570-L, 2.3 m x 1.2 m). Tanks were supplied with flow-through well-water at a flow rate of approximately 4.5 L/minute, resulting in a turnover rate of once every 28 hours. Each tank was a different temperature treatment (i.e. 27, 31, or 35°C) that covered the historical summer temperatures from commercial ponds in the Mississippi Delta, based upon data provided by Dr. E.L. Torrans of the Thad Cochran National Warmwater Aquaculture Center (TCNWAC; Stoneville, MS). The target temperature for the 27°C treatment was obtained using an in-line water chiller (CY-6 Cyclone In-Line

Water Chiller, Aqua Logic Inc., San Diego, CA), whereas the 31°C and 35°C treatments used submersible 1,700-watt titanium heaters (Process Technology Inc., Mentor, Ohio). Insulated covers (Dow Inc., Midland, MI) were placed on the tanks to aide in temperature maintenance as well as provide shade for the catfish. Covers were over half of the tank and allowed ambient light.

Fish were acclimated to treatment temperatures by increasing temperatures 1°C / day until the desired treatment temperature was reached and held for two weeks prior to experiments. During this time, catfish were fed daily at 0900 the same diet described previously to satiation. Photoperiod during acclimation and experiments was set at 14-hour-light and 10-hour-dark which is typical for the facility coordinates (33° 27' 45" N, 88° 49' 12" W) in July/August.

Water quality and cleaning

Water quality (Table 3.1) was measured 3 times a week using a colorimetric reader (Hach DR550, Hach Company Inc., Loveland, CO) for measuring total ammonia-nitrogen (TAN) and nitrite (NO₂⁻). Water pH was measured using a pH meter and glass electrode (pH10N, YSI Inc., Yellow Springs, OH) and dissolved oxygen concentrations were measured using an optical sensor and DO meter (YSI ProODO, YSI Inc., Yellow Springs, OH). Un-ionized ammonia (NH₃) was calculated using TAN, temperature, and pH. Tanks were siphoned three times a week to remove out fecal waste and feed, and scrubbed every other week to remove algae.

Table 3.1 Water quality values (mean \pm SE) recorded in holding tanks during active and resting metabolic studies, $n = 48$. Study occurred from July-November 2011, Mississippi State, MS.

Temp (°C)	TAN ¹ (mg/L)	NH ₃ (mg/L)	Nitrite (mg/L)	pH	DO ² (mg/L)	Saturation (avg. %)
27	0.16 \pm 0.03	0.01 \pm 0.00	0.03 \pm 0.01	7.9 \pm 0.12	5.80 \pm 0.15	73.0
31	0.13 \pm 0.03	0.01 \pm 0.00	0.04 \pm 0.01	8.0 \pm 0.11	5.84 \pm 0.17	78.8
35	0.15 \pm 0.05	0.02 \pm 0.01	0.03 \pm 0.01	8.2 \pm 0.17	6.00 \pm 0.17	86.7

¹Total ammonia-nitrogen

²Dissolved oxygen

Resting metabolism protocol

Resting metabolic rates were measured in an intermittent-flow, 25-L lexan respirometry chamber with a fiber-optic oxygen probe (DAQ-PAC-F1X, Loligo Systems, Tjele, Denmark) controlled by computer software (AutoResp™ version 2.0.0, Loligo Systems, Tjele, Denmark). One temperature treatment (27, 31, or 35°C) was observed per day, in which fish were not fed 24 hours prior to testing to ensure a post-absorptive state. An insulated rectangular 500-L, 3.05 m x 0.61 m x 0.61 m fiberglass tank (Model MT-1204, Frigid Units Inc., Toledo, OH) held the respirometry chamber and served as the location for experimental runs. The holding tank had been set to the desired treatment temperature using a submersible titanium heater (Process Technology Inc., Mentor, Ohio) the previous afternoon and allowed to stabilize overnight. An air stone maintained dissolved oxygen near saturation.

Pilot studies were conducted to determine necessary flush/recirculation times to measure catfish aerobic metabolism in the respirometry chamber and to determine

acclimation times based on behavior and stabilization in metabolic rate. For experiments, two catfish were randomly chosen from their circular holding tank and measured for total length (mm), standard length (mm), and wet weight (g) (Table 3.2) and transported to the rectangular testing tank the morning of testing. Fish were acclimated to the rectangular testing tank for 30 minutes at the end of which one fish was gently netted and nudged inside the respirometry chamber for an additional 30-minute acclimation time prior to starting the experiment. An egg-crate screen positioned above the respirometry chamber on the holding tank provided shade for the chamber as it was found to be a preferential refuge for the catfish during pilot studies and assisted in calming fish which was determined by reduction in swimming activity. Furthermore, the screen facilitated unobtrusive observation of fish for tailbeat frequency/gill ventilation frequency counts by shielding outside stimuli as well as to ensure the catfish was in a non-active state.

Each measurement cycle of the metabolic experiment lasted 720 seconds, which consisted of a 240-second flushing period, a 60-second interim period, and a 420-second measuring period during which oxygen consumption (MO_2) was recorded at the end of the cycle. Additionally, tailbeat frequency and gill ventilation frequency were visually counted midway through the measurement period for one, 30-second interval for each variable. This cycle was repeated three times and means values from the three final MO_2 readings as well as tailbeat and gill ventilation frequencies were used for statistical analyses. Upon completion of testing, the tip of the left pelvic fin was clipped as an identifying mark to ensure a unique fish was tested each time. From each of the three temperature treatments, six catfish were used totaling 18 fish. At the conclusion of each testing day, thorough rinses of the chamber, sensors, and tubes were done using de-

ionized water. A blank run of the oxygen chamber, without a catfish present, was conducted midway through the experiment after nine fish had been tested ensuring no background microbial respiration was occurring during experimental runs.

Active metabolism protocol

Each day prior to experiments, one temperature treatment (27, 31, or 35°C) was chosen, and all catfish in the designated treatment were not fed 24 hours prior to the start of swimming tests to ensure a post-absorptive state following Shrable et al. (1969). These catfish were transferred to a separate insulated rectangular (500-L, 3.05 m x 0.61 m x 0.61 m) tank (Model MT-1204, Frigid Units Inc., Toledo, OH) prior to testing. The holding tank had been set to the desired treatment temperature using a submersible titanium heater (Process Technology Inc., Mentor, Ohio) the previous afternoon and allowed to stabilize overnight. An air stone constantly delivered oxygen providing 100% oxygen saturation.

The morning of testing, two fish were randomly chosen from a treatment tank and placed into the separate rectangular holding tank. During this time, an insulated 100-L Blazka-type swim flume (Blazka et al., 1960) (Figure 3.1) was filled with treatment water and oxygen saturation was checked with a galvanic oxygen probe (Model MINI-DO, Loligo Systems, Tjele, Denmark) to ensure 100% saturation. Fish were removed from the holding tank by net, and quickly (< 10 seconds) placed into the flume to minimize aerial exposure time. Once the flume was sealed, fish were acclimated at a velocity of 10 cm/second for 1 hour. After 1 hour, the oxygen saturation percentage was recorded as a starting point for active metabolism calculations. Velocity was raised smoothly and

gradually (< 1 minute) to the final desired setting at 60 or 70 cm/second. Rotation among the three treatments occurred with regularity to ensure no single temperature treatment was starved for > 24 hours.

Water velocities used for endurance/active metabolic tests were based upon paddlewheel aerator currents measured by Dr. Craig Tucker at TCNWAC. A pilot study was conducted to observe catfish swimming endurance in a flume at a range of current velocities (e.g. 20-70 cm/second) taken from the TCNWAC data. This pilot study showed catfish sustained exercise without fatigue for 200 minutes at velocities < 70 cm/second, but at 70 cm/second fish fatigued in < 200 minutes. As active metabolism is defined as the maximum sustained rate for steady swimming (Fry 1971), active aerobic metabolic rate measurements were conducted at 60 cm/second, and time to fatigue was measured at 70 cm/second.

Calibration of the flume was conducted with a flow meter (Flo-Mate Model 2000, Hach Company Inc., Loveland, CO). A limit of 200 minutes was set for the experimental runs based upon Beamish's (1978) definition of long periods of sustained swimming performance. Tailbeat frequencies and gill ventilation frequencies were tallied for 30 second intervals at 10, 30, 60, and 120 minutes after the start of the velocity trial.

To prevent a significant oxygen drop during the experiment, periodic flushing of the flume occurred when *in situ* oxygen levels fell below 85% saturation. This was achieved using the same temperature treated water from the adjacent rectangular holding tank. Water was pumped to a head tank, where it was gravity fed through vinyl hosing to the outer chamber of the flume. Flushing occurred at the experimental velocity and generally lasted < 15 minutes, ceasing when DO concentrations were near saturation (e.g.

> 99.5%). To determine if microbial respiration affected oxygen readings, blank runs of the flume, in which no fish were present, occurred weekly. During a blank run, water velocity was sustained at 70 cm/second and oxygen saturation was recorded for 200 minutes, identical to experiments with fish. Following each blank run, the flume was treated with a dilute 20 mg/L bleach solution overnight, which helped prevent bacterial respiration.

The experiment concluded when the 200-minute limit was reached or after impingement. Impingement was determined as the immobilization/cessation of swimming effort while in prolonged (> 5 seconds) caudal, ventral, or dorsal contact with the rear of the chamber. Upon first impingement, time was stopped and the velocity was reduced to 0 cm/second. Fish were given a 10-second recovery time prior to returning smoothly and gradually (< 1 minute) to the test velocity, after which time was continued. The experiment was stopped after three impingements, and the final oxygen saturation was recorded. Fish were then measured for total length, standard length, girth, and wet weight (Table 3.2). Catfish were then marked by a fin clip and released back into their respective treatment tanks. Six unique fish per treatment were used for 60 cm/second and ten unique fish per treatment were used for 70 cm/second velocity tests. Solid blocking occurs where an object impedes flow in the flume cross-section and may increase flow velocity surrounding the object (Bell and Terhune 1970). As all fish were < 10% of the cross-sectional area of the flume, no correction factor for solid blocking was needed.

Table 3.2 Mean (\pm SE) wet weights and total lengths (TL) for juvenile channel catfish (*Ictalurus punctatus*) used for active and resting metabolic studies. Differing letters indicate statistical differences (one-way ANOVA, Tukey-Kramer HSD, $P < 0.05$). Study occurred from July-November 2011, Mississippi State, MS.

Velocity (cm/second)	Temp (°C)	N	Wet Weight (g)	TL (mm)
70	27	10	206.0 \pm 15.3 b	284.5 \pm 6.7 bb
	31	10	226.2 \pm 21.9 b	291.4 \pm 10.5 bb
	35	10	132.8 \pm 14.2 a	252.4 \pm 8.0 aa
60	27	6	177.2 \pm 13.8 A	283.2 \pm 6.0 BB
	31	6	170.6 \pm 32.7 AB	270.5 \pm 12.4 BB
	35	6	91.0 \pm 13.3 B	234.0 \pm 8.1 AA
0 (resting)	27	6	380.5 \pm 23.6	338.2 \pm 7.5
	31	6	305.9 \pm 42.2	317.2 \pm 14.5
	35	6	285.0 \pm 49.1	295.0 \pm 16.9

Oxygen consumption

Oxygen consumption (MO_2) was calculated according to Cech (1990):

$$MO_2 = \left[\frac{(cO_{2i} - cO_{2f})}{hour} \right] / fw \cdot V \quad (3.1)$$

where MO_2 = oxygen consumption rate (mgO₂/kg of fish/hour); cO_{2i} = initial oxygen content (mg/L) at 100% saturation; cO_{2f} = final oxygen content (mg/L); *hour* = duration of test in hours; *fw* = fish weight in kg; and *V* = volume of swim chamber in liters.

Temperature coefficient

A temperature coefficient (Q_{10}), measuring the sensitivity of aerobic metabolic rate to temperature, was calculated according to Schmidt-Nielsen (1983):

$$Q_{10} = (V_2/V_1)^{10/T_2-T_1} \quad (3.2)$$

where V_2 and V_1 are MO_2 values at respective temperatures of T_2 and T_1 .

Scope for activity

Scope for activity was calculated from the difference between resting and active metabolic rates for each temperature treatment (Fry 1947).

Statistical analyses

Normality and homogeneity of variance for active and resting metabolic rates, wet weight, tailbeat frequency, and gill ventilation frequency values were determined by the Shapiro-Wilk and Levene's tests using JMP (version 9.0.2, SAS Institute, Cary, NC). A one-way analysis of variance (ANOVA) was used to analyze metabolic rates and wet weight for statistical significance, whereas a two-way ANOVA with factors of time and temperature treatment was used to analyze gill ventilation frequency and tailbeat frequency. Nonparametric analyses were observed with a Kruskal-Wallis test. Analysis of covariance (ANCOVA) was used to determine if wet weight was a covariate of oxygen consumption rate. Means comparisons were conducted with Tukey-Kramer Honestly Significant Difference tests and student's t -tests. Statistical significance was $\alpha = 0.05$ and all values are presented as mean \pm standard error (SE).

Results

Fish weight and size

Catfish were in holding tanks at treatment temperatures for a period of 4 months during acclimation, active, and resting metabolic experiments. Although they were introduced into holding tanks at similar sizes, by the time experiments were completed catfish experienced growth differences among temperature treatments. These growth differences support the results of Chapter II, where catfish held at the highest temperatures (31-35°C in Chapter II, 35°C here) grew more slowly in comparison to lower temperature treatments. Thus, size differences were unavoidable for fish acclimated for a long time period to the different temperature treatments. However, analysis of covariance for resting and active metabolic studies showed that differences in wet weight did not affect metabolism (catfish resting at 0 cm/second: $P = 0.70$; catfish exercised at 60 cm/second: $P = 0.79$; catfish exercised at 70 cm/second: $P = 0.18$). Survival of catfish (Table 3.3) in this study was recorded but not analyzed statistically.

Table 3.3 Survival rates of juvenile channel catfish (*Ictalurus punctatus*) in growth (Chapter II) and metabolism studies (Chapter III). Growth study occurred from August-October 2010, while the metabolism study occurred from July-November 2011, Mississippi State, MS.

	Growth			Metabolism		
	23-27°C	27-31°C	31-35°C	27°C	31°C	35°C
Mortalities	0	1	9	7	1	17
% Survival	100	99.2	92.5	76.7	96.7	57.5

Resting oxygen consumption, tailbeat frequency, and gill ventilation frequency

Resting oxygen consumption for catfish held in 27°C was (mean \pm SE) 140.7 ± 16.4 mgO₂/kg fish/hour. Catfish held at 31°C consumed 173.2 ± 16.4 mgO₂/kg fish/hour, whereas a consumption rate of 204.9 ± 16.4 mgO₂/kg fish/hour was recorded for catfish held in the 35°C treatment (Figure 3.2). These values differ significantly ($P = 0.037$, one-way ANOVA) and indicate a difference in resting metabolic rates between the three temperature treatments. Oxygen consumption for fish in the 27 and 35°C treatments differed significantly ($P = 0.036$), whereas there were no significant differences for catfish in the 27 and 31°C treatments and catfish in the 31 and 35°C treatments. The temperature coefficient (Q_{10}) was 1.60 between 27 and 35°C, 1.68 between 27 and 31°C, and 1.51 between 31 and 35°C.

Tailbeat frequencies were not normally distributed, and were analyzed using a nonparametric test (Kruskal-Wallis test, $P = 0.369$) and found to be not different for fish among temperature treatments. The reduced tailbeat frequency values among fish held at the three temperature treatments shows that activity within the respirometry chamber was considered as a resting state whereas the lack of difference among treatments shows no activity bias was present allowing for comparison. Gill ventilation frequencies were not different in the fish among temperature treatments.

Active oxygen consumption, tailbeat frequency, gill ventilation frequency, and time to fatigue

Wet weight was not determined to be a significant covariate to metabolism (ANCOVA). Active oxygen consumption for catfish exercised at 60 cm/second and held

in 27°C was (mean \pm SE) 292.5 ± 38.7 mgO₂/kg fish/hour. Catfish held at 31°C consumed 357.0 ± 34.1 mgO₂/kg fish/hour, whereas a consumption rate of 443.7 ± 38.7 mgO₂/kg fish/hour was recorded for catfish held in the 35°C treatment while being exercised at 60 cm/second (Figure 3.2). There were significant differences ($P = 0.019$) among fish held in the different treatments. Oxygen consumption was different between fish held in the 27 and 35°C treatments ($P = 0.015$), whereas catfish in the 27 and 31°C and 31 and 35°C treatments were not different. The temperature coefficient (Q_{10}) was 1.68 between 27 and 35°C, 1.65 between 27 and 31°C, and 1.72 between 31 and 35°C.

Active oxygen consumption for catfish exercised at 70 cm/second and held in 27°C was (mean \pm SE) 426.5 ± 79.6 mgO₂/kg fish/hour. Catfish held at 31°C and exercised at 70 cm/second consumed 611.7 ± 153.0 mgO₂/kg fish/hour, whereas a consumption rate of 598.9 ± 142.1 mgO₂/kg fish/hour was recorded for catfish held in the 35°C treatment (Figure 3.3). These data were not normally distributed (Shapiro-Wilk, $P = 0.001$) and therefore were analyzed nonparametrically and did not differ significantly (Kruskal-Wallis, $P = 0.393$).

Gill ventilation frequency in catfish swum at 60 cm/second was significant at 60 and 120 minutes, where catfish in the 35°C temperature treatment had the highest values (two-way ANOVA, student's t -test, $P < 0.05$) (Figure 3.4). Also, there was a main effect of temperature as gill ventilation frequency differed significantly among all treatments (Figure 3.5). Tailbeat frequency in catfish swum at 60 cm/second had no differences between treatments (two-way ANOVA, student's t -test) (Figure 3.6).

At 70 cm/second, several fish across all treatments fatigued prior to the initial 10-minute measurement and were not used in ventilation frequency or tailbeat frequency

analyses but data were used in assessment of time to fatigue. Frequencies of tailbeat (Figure 3.7) and gill ventilation (Figure 3.8) of catfish held at 35°C were significantly higher at 10 minutes, whereas catfish held in 27 and 35°C were higher at 30 minutes (two-way ANOVA, student's *t*-test, $P < 0.05$). There were no differences at 60 or 120 minutes. A main effect of temperature was found between temperature and gill ventilation frequency and tailbeat frequency with catfish in the 27 and 35°C treatments having greater values than catfish in the 31°C (two-way ANOVA, student's *t*-test, $P < 0.05$).

All fish swum at 60 cm/second did not fatigue for the 200-minute testing period suggesting temperature was not a significant factor in endurance at this velocity. Conversely, catfish swum at 70 cm/second fatigued (mean \pm SE) after 106.4 ± 31.3 minutes for fish held at 27°C, 54.9 ± 27.5 minutes for catfish in the 31°C treatment, and 44.2 ± 23.3 minutes for those held in the 35°C treatment, suggesting velocity was significant in determining endurance at this temperature (Figure 3.9).

Scope for activity

Taking the differences in oxygen consumption rates of catfish exercised at 60 cm/second and resting (0 cm/second) catfish, the scope for activity (Figure 3.10) for catfish in the 27°C treatment was 151.8 mgO₂/kg fish/hour. Catfish held at 31°C differed by 183.9 mgO₂/kg fish/hour, whereas catfish held at 35°C differed by 238.8 mgO₂/kg fish/hour. Scope for activity for catfish exercised at 70 cm/second (Figure 3.11) and held at 27°C was 285.8 mgO₂/kg fish/hour. Catfish held at 31°C differed by 423.5 mgO₂/kg fish/hour, whereas catfish held at 35°C differed by 394.0 mgO₂/kg fish/hour.

Discussion

The goal of this study was to determine juvenile channel catfish resting and active metabolic rates, scope for activity, and time until fatigue during warm summer conditions in the Mississippi Delta and the southeastern U.S. As with other teleosts, resting and active metabolic rates increased with increasing temperatures. The results of this study show the potential stress-inducing effects of high water temperatures on metabolic rates and exercise endurance. The water temperatures examined are reflective of current seasonal weather patterns and also are within range of those predicted under current climate change models.

While fish weight differed by temperature as the experiments progressed, wet weight was not determined a significant covariate to metabolism, and all fish except those used in the 27°C resting treatment fell in the range of “large stocker” size as recognized by the USDA National Agriculture Statistics Service (2011).

Resting metabolism

Resting oxygen consumption rates ranged from 140-204 mgO₂/kg/hour, which were similar to Moss and Scott's (1961) findings of 77-193 mgO₂/kg/hour for 12-103 g channel catfish acclimated to 25, 30, and 35°C. Beecham (2004) found a slightly higher resting metabolic rate (299 mgO₂/kg/hour) for 80-g channel catfish fingerlings and a comparable rate (149 mgO₂/kg/hour) for 90-g blue catfish (*Ictalurus furcatus*) fingerlings held at 20°C.

Oxygen consumption rates are similar to those in other species at comparable sizes including: 10.8-422.6 g southern catfish (*Silurus meridionalis*) at 30°C (Xiaojun

and Ruyung 1990) and 400-500 g turbot (*Scophthalmus maximus*) at 22°C (Mallekh and Lagardere 2002). Although gill ventilation frequency in the 35°C treatment was not different in comparison to other treatments, this is likely a result of the sample size ($n = 6$ per treatment).

Active metabolism

Active oxygen consumption rates at 60 cm/second ranged from 292-443 mg O₂/kg/hour. When forced to swim at 70 cm/second, oxygen consumption rates ranged from 426.5-611.7 mg O₂/kg/hour. These values are similar to active metabolic rates in juvenile 80 g channel catfish and blue catfish (*Ictalurus furcatus*) in studies from Beecham (2004) and Bartlett (1998). The active metabolic rates in this study are also similar to those measured in a salmonid, rainbow trout (*Oncorhynchus mykiss*), at similar body weights in water temperatures of 26-30°C (Rodnick et al. 2004). Conversely, these oxygen consumption rates were substantially greater than those measured in juvenile (~20 g wet weight) southern catfish (*Silurus meridionalis*) exercised at 33°C (Pang et al. 2010). Tailbeat frequency did not change, and surprisingly, swimming endurance did not decrease in comparison to the 27 and 31°C treatments. Body size may have been a factor, as tailbeat frequency typically decreases and time to fatigue typically increases with body size (Hammer 1995).

A notable result of the swimming endurance test was that all fish, regardless of temperature did not fatigue after 200 minutes at 60 cm/second. This contradicts Beecham et al. (2007) who found that smaller, 80-g channel catfish fatigued at velocities above 50 cm/second in 19-22°C temperature environments. Additionally, these findings differ from

previous studies on swimming endurance in southern catfish (*Silurus meridionalis*) fingerlings at temperature ranges of 10-30°C, which peak at 25°C and decline with increasing temperature (Zeng et al. 2009). Differing results are difficult to explain but may be due to the larger body sizes of channel catfish in the present study, as swimming capabilities are generally related to body size (Hammer 1995).

Although most fish fatigued at 70 cm/second, at least two catfish from each temperature treatment were able to endure the 200 minute experiment at this velocity showing that some channel catfish of the size tested (188.3 ± 12.2 g) have the capability to swim faster than the maximum velocity recorded for a paddlewheel aerator current within this timeframe. However, endurance beyond 200 minutes is still undefined.

Scope for activity

There was no reduction of metabolic scope as temperature increased at 60 cm/second, which generally occurs as fish experience temperatures outside of optimal conditions (Fry 1971; Neill and Bryan 1991). Because metabolic scope decreases when fish are exposed to near-lethal temperatures, it may be surmised that catfish in this experiment were not outside their optimal temperature range when exercised at this velocity. However, this is not supported by mortality results of this experiment and the results of the previous growth experiment. One explanation may be that as body weight increases, weight-specific oxygen consumption rates decrease. Thus, metabolic rates are significantly higher in small fish compared to larger fish. In the 35°C temperature treatment, there was a size difference between channel catfish measured for resting (wet weight: 91 ± 13.3 g) and active (wet weight: 285 ± 49.1 g) oxygen consumption rates.

Size difference was also present between the other treatments. However, body size was not found to be a significant covariate of metabolism.

At a water velocity of 70 cm/second, there was a reduction in metabolic scope for activity in catfish held at 35°C in comparison to catfish held at 31°C. This supports the scope for activity model defined by Fry (1947, 1971) and illustrated in sockeye salmon (*Oncorhynchus nerka*) in Brett (1964), which predicted decreasing metabolic scope as temperatures increased outside a range of thermal preference. Fry (1971) and Brett (1979) indicate that scope for activity is better calculated from maximum sustained swimming speeds. This is supported in tests at 60 cm/second, which had no reduction of scope in non-fatiguing fish as well as the 70 cm/second tests, which did show a reduction of scope in fatiguing fish. Therefore, the 70 cm/second data provides a clearer picture of metabolic scope.

Q_{10}

Q_{10} 's were similar for resting and active metabolic rates at 60 cm/second and ranged 1.5-2. Wells (1932) and Moss and Scott (1961) determined that Q_{10} values near 2 are characteristic of sufficiently acclimated and normally performing fish. Further, the lack of a significant increase in Q_{10} at 35°C may indicate that this temperature was not challenging. However, this conflicts with observed mortality results of this experiment and the results of the previous growth experiment. Additionally, this temperature is near the upper lethal limit of acute temperature tolerance studies for channel catfish (Allen and Strawn 1968). Q_{10} 's at 70 cm/second velocity tests were much different, with increased metabolic activity between 27 and 31°C ($Q_{10} = 2.46$), and a large decrease between 31

and 35°C ($Q_{10} = 0.95$). This indicates a considerable, potentially damaging, drop in metabolic function (Hochachka and Somero 2002) and illustrates the reduction of scope for activity. The lack of difference in Q_{10} values between resting and 60 cm/second active metabolic rates may be due to the relatively minimal challenge of the 60 cm/second swimming endurance tests for the duration of time measured. In support, all fish were able to swim for the entire 200 minutes at 60 cm/second. In contrast, and reflected by the reduced Q_{10} and scope for activity, most channel catfish exercised at 70 cm/second fatigued prior to the 200 minute swimming period.

Conclusions

Considering the results of the previous chapter, as catfish exercised at 60 cm/second in 35°C water have greater metabolic rates than at cooler temperatures (27 and 31°C), this does not translate to increased feeding and growth. The implications of reduced feeding and increased sustained respiration as a result of thermal stress come in combination with high energetic costs. These costs are illustrated when observing decreases in metabolism, scope for activity, Q_{10} , and swimming endurance in catfish exercised at 70 cm/second in water temperatures of 35°C. Aside from metabolic complications, other studies have found that these conditions can result in increased lipid production, reduced storage quality, and decreased fillet yields (Andrews and Stickney 1972; Minchew et al. 2007). An analysis of stroke volume or gill ventilation efficiency would aid in better defining thermally-challenged respiration. Moreover, future research should focus on the influences of other sub-optimal water quality stressors such as hypoxia and nitrogenous wastes at high temperatures.

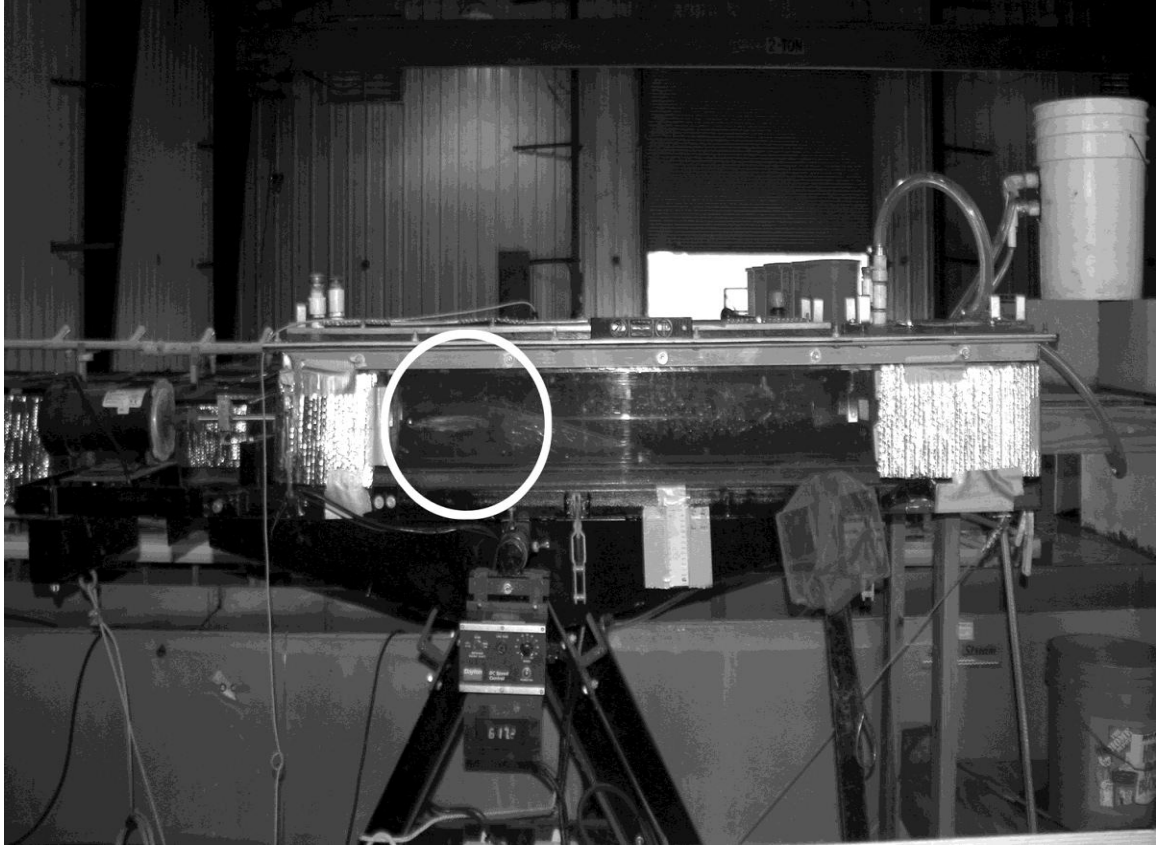


Figure 3.1 Picture of insulated, 100-L Blazka-type swim flume used for aerobic metabolic rate studies for juvenile channel catfish (*Ictalurus punctatus*) exercised at water velocities of 60 and 70 cm/second at water temperatures of 27, 31, and 35°C. Note catfish outlined in white ring. Study occurred from July-November 2011, Mississippi State, MS.

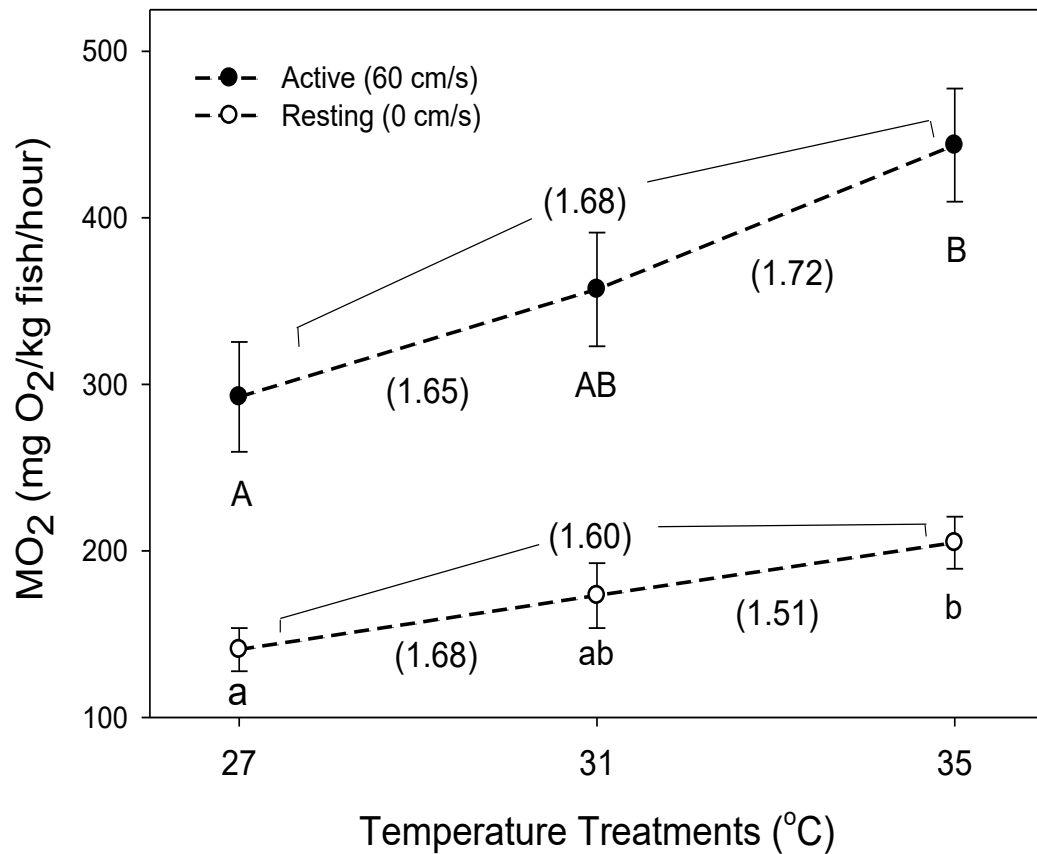


Figure 3.2 Comparison of active and resting mean (\pm SE) MO₂ rates for juvenile channel catfish (*Ictalurus punctatus*). Different letters indicate significant differences within each temperature treatment (one-way ANOVA and Tukey's HSD, $P < 0.05$, $n = 6$ fish per treatment). Parenthetical numbers represent Q_{10} values. Study occurred from July-November 2011, Mississippi State, MS.

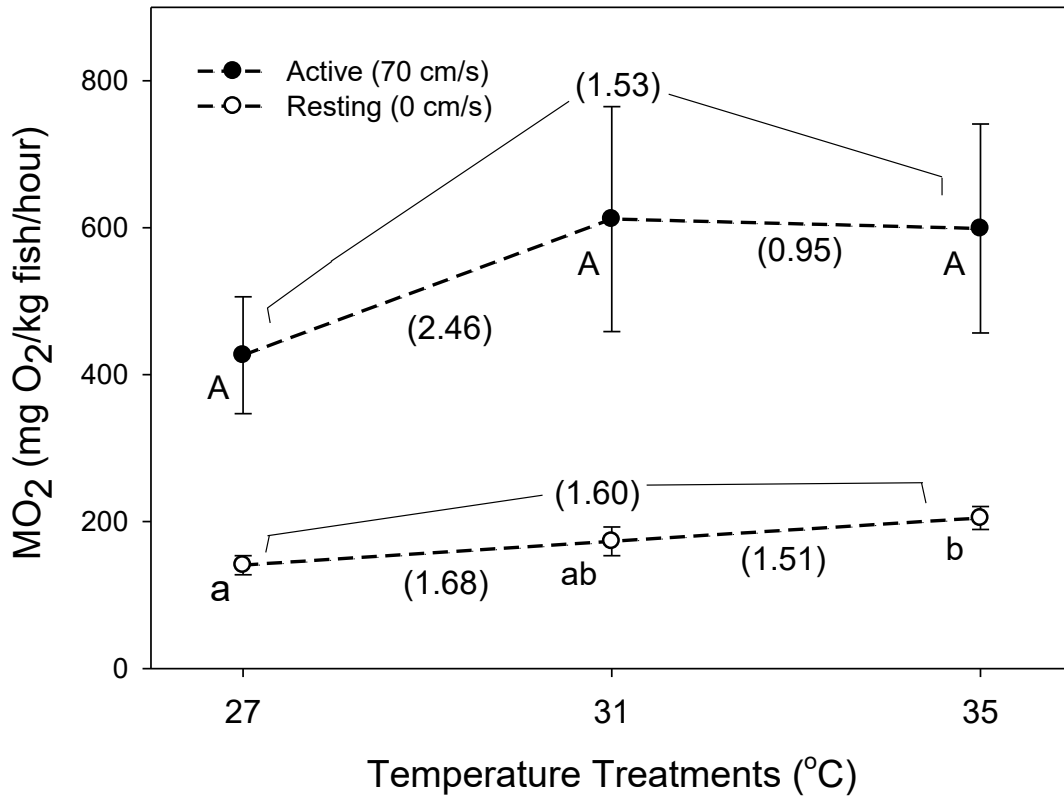


Figure 3.3 Comparison of active and resting mean (\pm SE) MO₂ rates for juvenile channel catfish (*Ictalurus punctatus*). Different letters indicate significant differences within each temperature treatment (Kruskal-Wallis, one-way ANOVA and Tukey's HSD, $P < 0.05$, $n = 6-10$ fish per treatment). Parenthetical numbers represent Q_{10} values. Study occurred from July-November 2011, Mississippi State, MS.

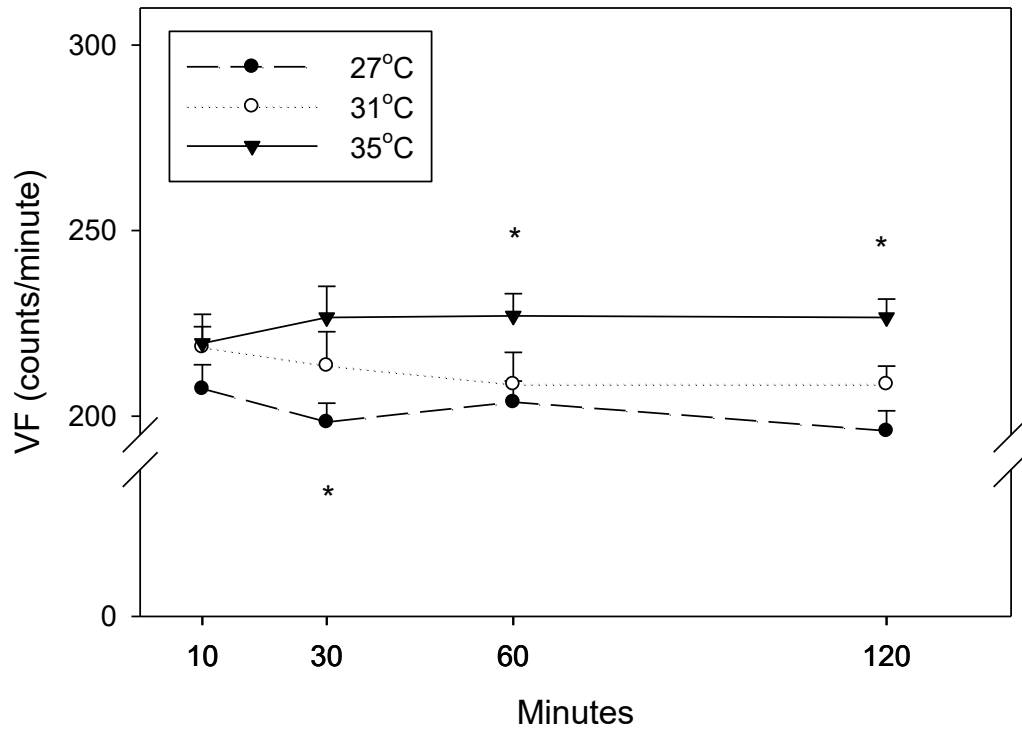


Figure 3.4 Juvenile channel catfish (*Ictalurus punctatus*) gill ventilation frequency (VF; counts/minute) during active aerobic metabolism experiments at 10, 30, 60, and 120 minutes of exercise at a water velocity of 60 cm/second. Asterisks on respective temperature line signify differences among the three temperature treatments at each time interval (two-way ANOVA, student's *t*-test, $P < 0.05$, $n = 6$ fish per treatment). Study occurred from July-November 2011, Mississippi State, MS.

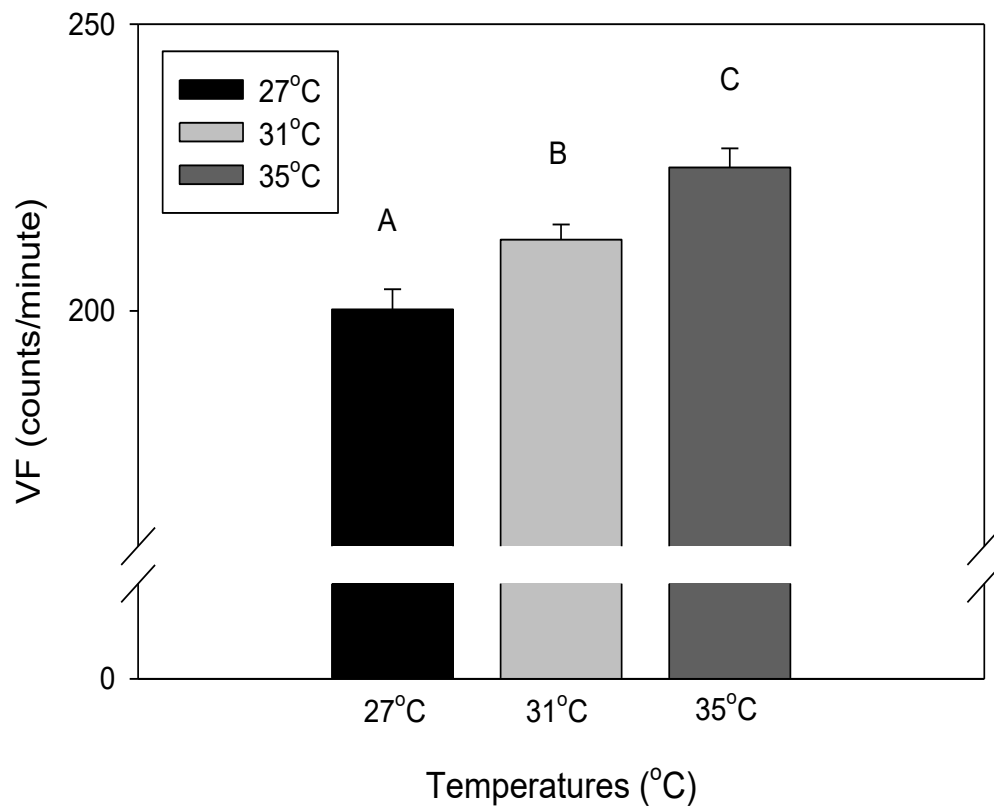


Figure 3.5 Mean (\pm SE) ventilation frequency (VF) for juvenile channel catfish (*Ictalurus punctatus*) swum at 60 cm/second. Different letters indicate significant differences (two-way ANOVA, Tukey-Kramer HSD, $P < 0.05$, $n = 6$ fish per treatment). Study occurred from July-November 2011, Mississippi State, MS.

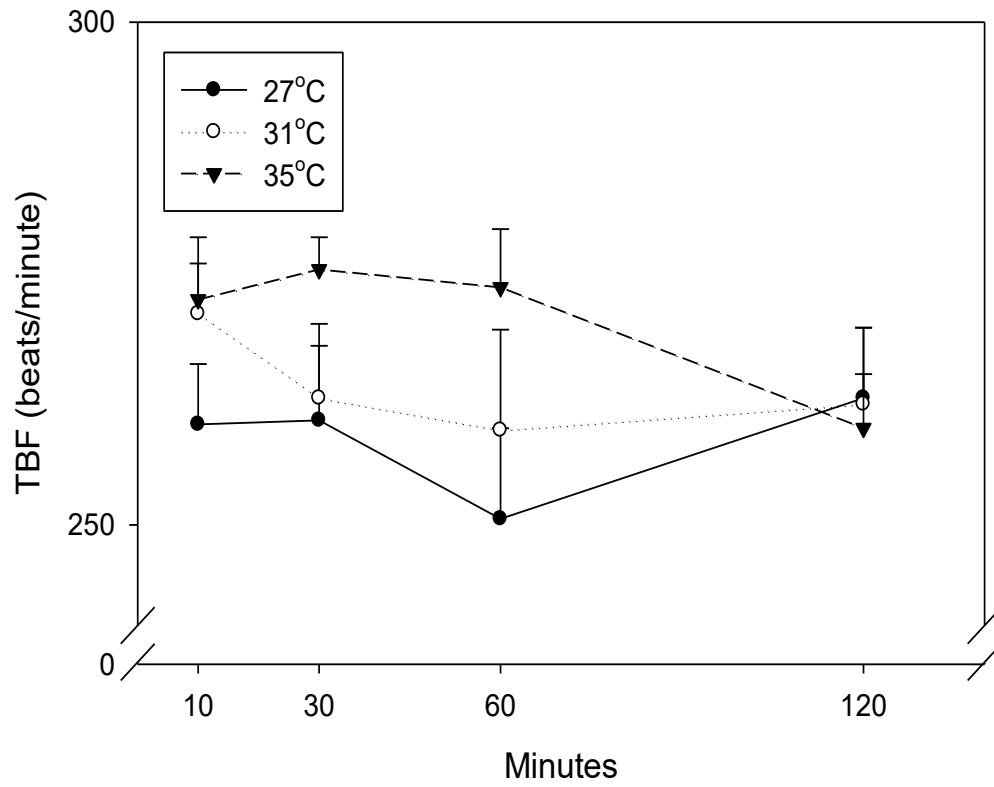


Figure 3.6 Juvenile channel catfish (*Ictalurus punctatus*) tailbeat frequency (TBF; beats/minute) during active aerobic metabolism experiments at 10, 30, 60, and 120 minutes of exercise at a water velocity of 60 cm/second. No significant difference was found among the three temperature treatments (two-way ANOVA, $P > 0.05$, $n = 6$ fish per treatment). Study occurred from July-November 2011, Mississippi State, MS.

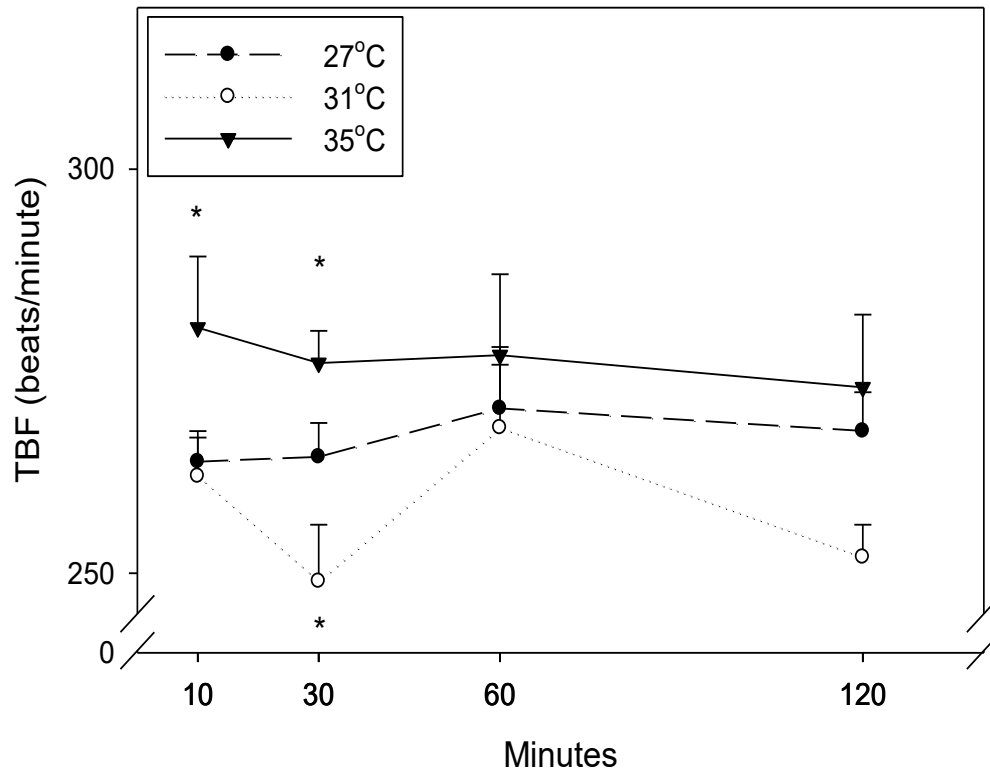


Figure 3.7 Juvenile channel catfish (*Ictalurus punctatus*) tailbeat frequency (TBF; beats/minute) during active aerobic metabolism experiments at 10, 30, 60, and 120 minutes of exercise at a water velocity of 70 cm/second. Asterisks on respective temperature line signify differences (two-way ANOVA, student's *t*-test, $P < 0.05$, $n = 2-7$ fish per treatment). Study occurred from July-November 2011, Mississippi State, MS.

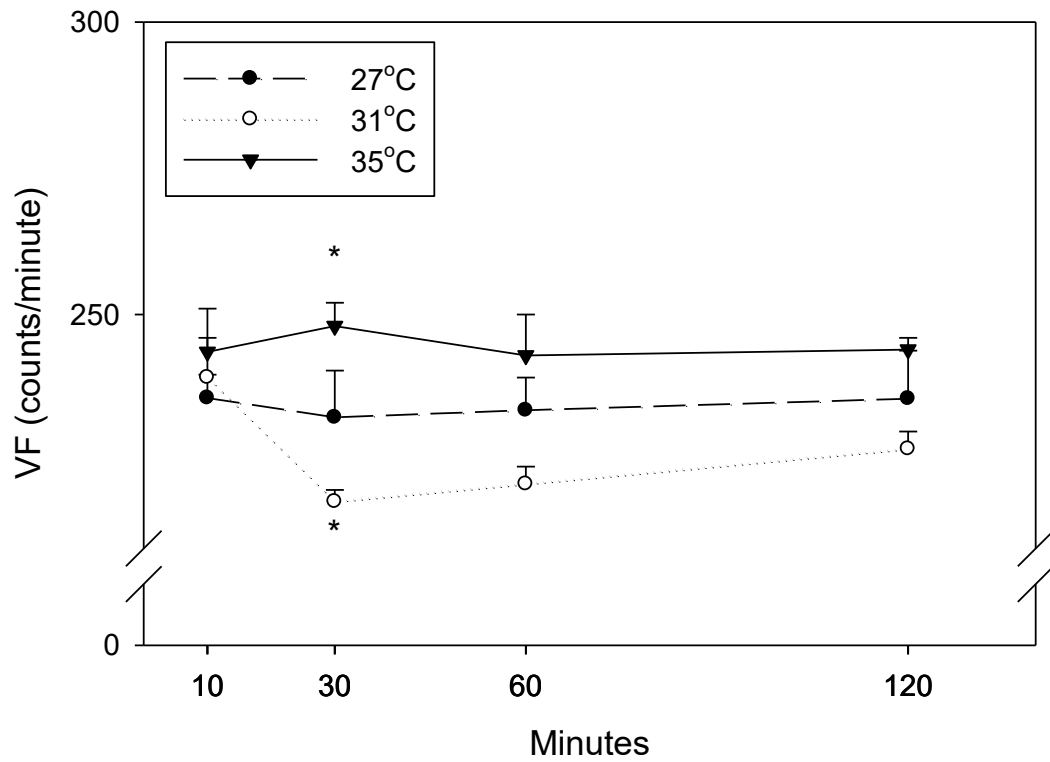


Figure 3.8 Juvenile channel catfish (*Ictalurus punctatus*) gill ventilation frequency (VF; counts/minute) during active aerobic metabolism experiments at 10, 30, 60, and 120 minutes of exercise and a water velocity of 70 cm/second. Asterisks on respective temperature line signify differences (two-way ANOVA, student's *t*-test, $P < 0.05$, $n = 2-7$ fish per treatment). Study occurred from July-November 2011, Mississippi State, MS.

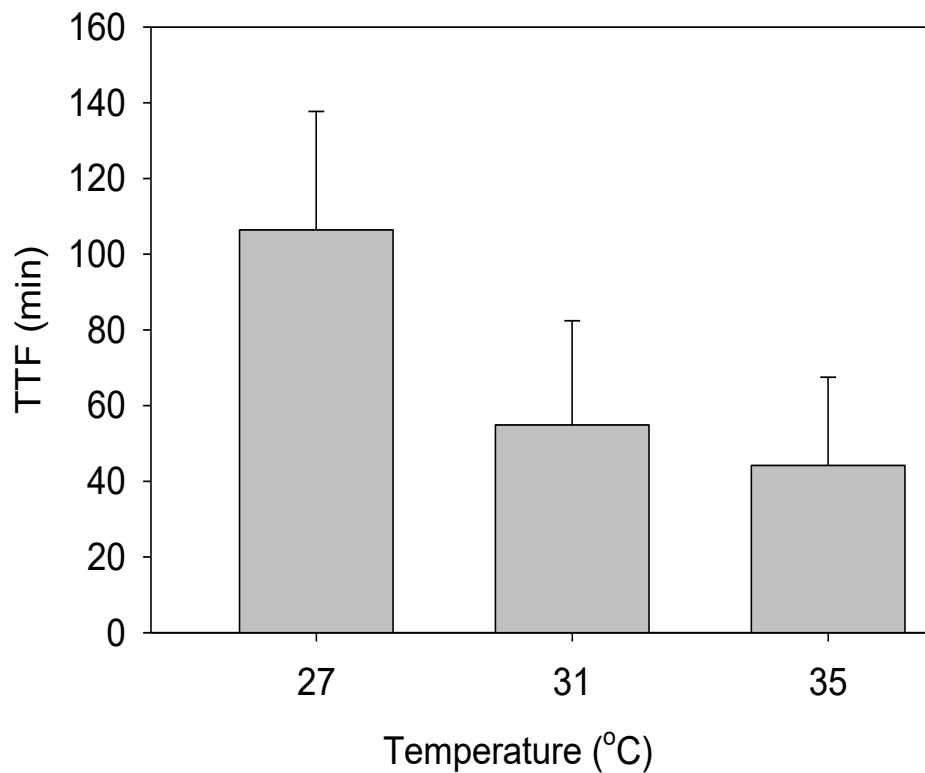


Figure 3.9 Mean (\pm SE) time to fatigue (TTF) in juvenile channel catfish (*Ictalurus punctatus*) swum at 70 cm/second and water temperatures of 27, 31 and 35°C. No significant difference was determined among the three groups (Kruskal-Wallis, $n = 10$ fish per treatment). Study occurred from July-November 2011, Mississippi State, MS.

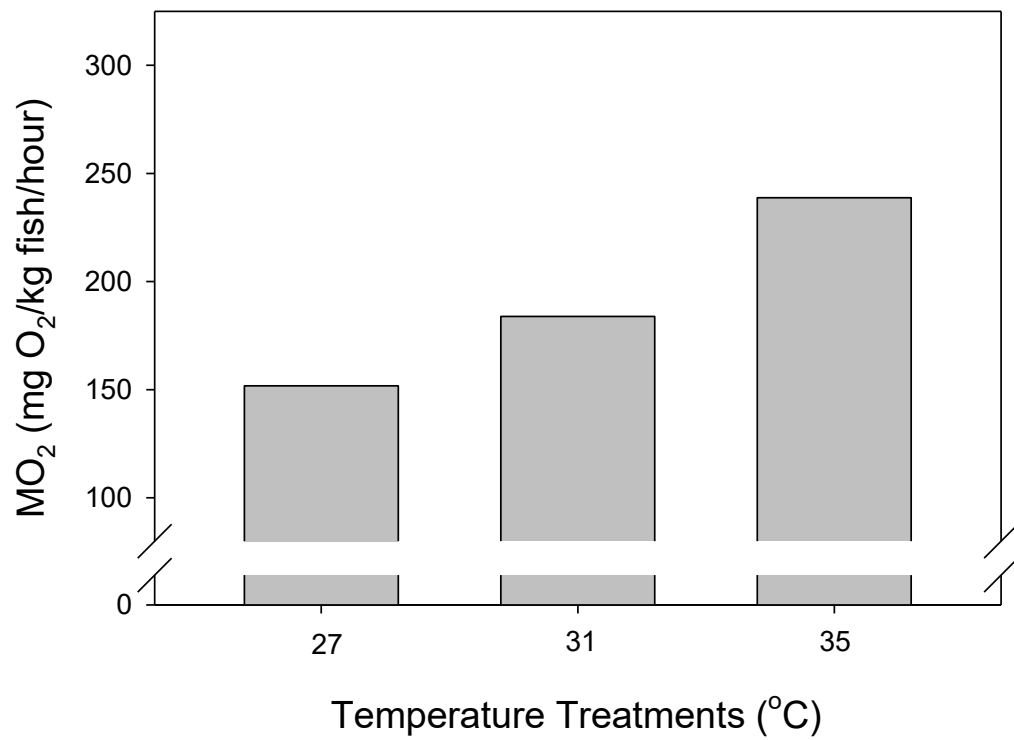


Figure 3.10 Channel catfish (*Ictalurus punctatus*) metabolic scope at water temperatures of 27, 31, and 35°C between water velocities of 0 and 60 cm/second. Study occurred from July-November 2011, Mississippi State, MS.

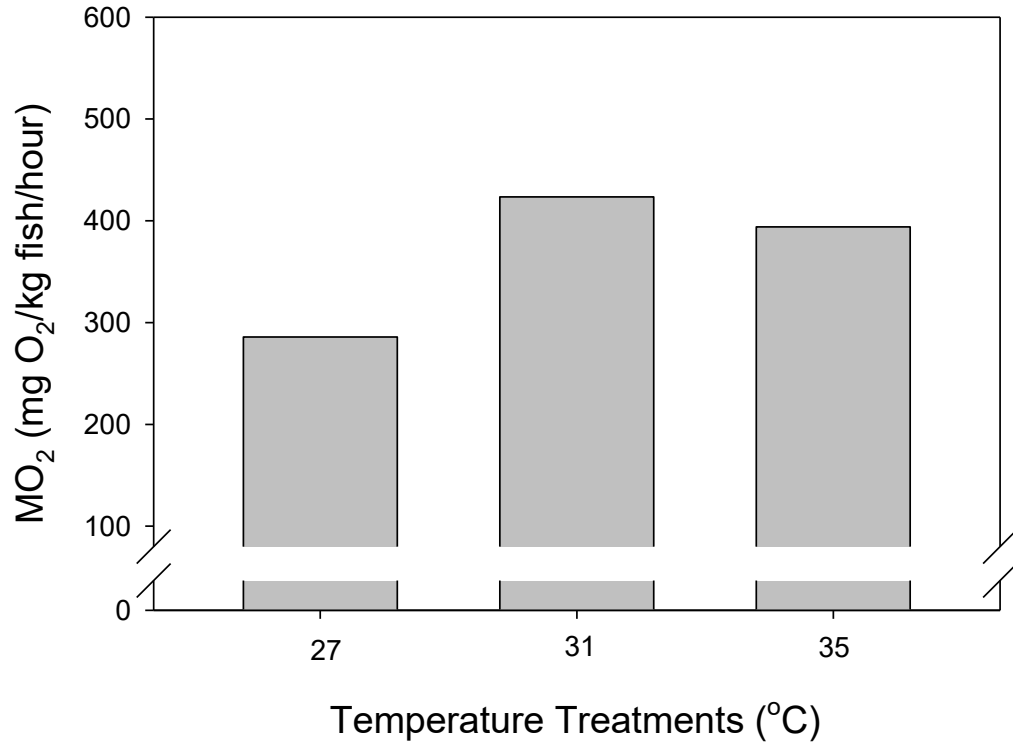


Figure 3.11 Channel catfish (*Ictalurus punctatus*) metabolic scope for activity at water temperatures of 27, 31, and 35°C between water velocities of 0 and 70 cm/second. Study occurred from July-November 2011, Mississippi State, MS.

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CHAPTER IV

SYNTHESIS

This research identifies and quantifies physiological responses of juvenile (size range: 234-380 mm, 91-380 g) channel catfish to seasonally-elevated water temperatures ranging from 23-35°C. Optimal temperatures for channel catfish feeding and growth occur at 27-31°C, which is supported by Andrews and Stickney (1972), Buentello et al. (2000) and Shrable et al. (1969). At higher temperatures, growth and feed consumption diminished concomitantly with increases in swimming activity. Aerobic metabolic rates were strongly influenced by temperature. However, swimming endurance at velocities of 60 and 70 cm/second did not differ between temperature treatments, indicating that juvenile channel catfish are strong swimmers, even when raised in a largely static environment.

The results of the growth experiment revealed that the 27-31°C temperature treatment had the greatest standard length, total length, and wet weight at the midpoint (week 4) and conclusion (week 8) of the experiment. Further, feed conversion rate was more efficient and specific growth rate was greater at this temperature treatment. Catfish at the 31-35°C treatment had the least survival rate (92%) of the three temperature treatments. The activity assessment found that catfish at the 31-35°C temperature treatment were significantly more active than fish in the cooler treatment temperature

groups. This may be a behavioral result of discomfort or seeking thermal refuge as described in Fry (1971) and further demonstrated in thermally stressed freshwater sculpin (*Cottus cognatus gracilis*) (Otto and Rice 1977) and mosquitofish (*Gambusia affinis*) (Cherry et al. 1976). Increased activity, coupled with decreased feed conversion rate are likely the causal factors for reduced growth in this group.

Temperature has a well-known relationship with metabolic rate (Johnston and Dunn 1987; Schurmann and Steffensen 1997). Active (60 cm/second and 70 cm/second) and resting (0 cm/second) aerobic metabolic rates differed among the three treatments in a direct relationship to ambient water temperature. A decrease in metabolism, scope for activity, swimming endurance, and Q_{10} in catfish exercised at 70 cm/second in water temperature of 35°C illustrates diminishing metabolic function for thermally stressed fish. Catfish exercised at 60 cm/second sustained the maximum 200 minutes of exercise, indicating the innate swimming ability that these fish have even when raised in static culture conditions. However, current-generating pond aerators may be on much longer than 200 minutes, and occur with reduced oxygen and greater nitrogenous waste concentrations, likely diminishing swimming endurance.

This research has important implications for aquaculturalists and natural resource managers. One implication is that feeding regimes should be reduced on hot summer days when overheating of ponds may occur, because fish may reduce feed intake in response to the stressful conditions. Furthermore, the reduction of feed on hot summer days will improve water quality which may otherwise diminish due to the decomposition of uneaten feed. Additionally, the concern over climate change, which may increase average water temperatures by 2°C or more in the next century (Murphy 1995; Johnston and Ball

1997), may potentially have long-term implications for channel catfish culture in the Southern U.S.

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